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# CELL PHYSIOLOGICAL STUDIES OF FROST RESISTANCE: A REVIEW

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The range of wild or cultivated plant species toward high latitude and altitude is largely determined by their resistance to frost. Not only winter killing is involved but also the effect of frost in limiting the length of the seasonal period of growth and photosynthesis. Survival of winter may depend in part on characters of the plant as a whole. In very cold climates the 'life forms' of the vegetation are largely such as to place the renewal buds under a cover of snow, dead leaves or earth. But such plants must also be able to endure a certain degree of frost, while trees and all plant parts which are without cover have to withstand the minimum air temperature of their climatic region. Frost resistance proper —which alone concerns us here—depends on the characters, morphological and physiological, of the individual cell.

Cellular resistance ranges from none at all to complete resistance, that is, to endurance of the lowest temperatures that can be produced. Some plants are always sensitive except usually in the seed stage. Others, members of the lower groups of plants, are highly resistant—not only to frost but also to desiccation—at all times. In most higher plants, however, the tissues possessing potential resistance are not actually resistant when in active growth. Thus tree twigs which may endure $-50°$ C. in winter without injury are killed by $-5°$ C. in summer. The process of acquiring resistance termed 'hardening' or 'hardening off' is normally induced by low temperature below a certain threshold but may also result from drought and other conditions which check growth.

The nature of the cell changes which take place during hardening and of the protection which they afford has been the subject of much research. More than 600 publications on the subject are cited in a recent critical review by Levitt (1941). Inquiry has mostly taken the line of correlating the power of resistance of different tissues with the chemical or colloidal properties of saps or extracts obtained from them. Differences in the colloidal state of the living protoplasm, however, are not necessarily preserved in dead material and differences in structure are lost. It is not surprising, therefore, that the hardening changes which have hitherto been revealed by biochemical methods are not invariable or essential ones.

The techniques of cell physiology which deal with living material have been applied to the problem by relatively few. Consequently the cellular studies made during recent years by the writer's associates (chiefly Levitt and Siminovitch) have had the advantage of a rather open field, and have made correspondingly rapid progress. It is found that resistance to frost rests largely on characters of organized protoplasm which only the study of living cells could reveal. Nevertheless, underlying all protoplasmic changes which can actually be observed there must be physico-chemical ones, the nature of which is no more than suggested by the data obtained on living cells. More direct knowledge of these must still be sought by biochemical methods so adapted perhaps as not to alter too much the

critical characters of the protoplasmic colloids. The results now obtained by the study of living cells point the direction in which the chemical study should proceed, and a start has been made in following that lead.

The present review deals mainly with the work of the McGill group but also attempts an estimate of the present status of the whole problem based on this work and on Levitt's review of the literature. In view of the comprehensive list of references in the latter monograph only the publications of the McGill workers and a few others pertinent to the discussion are quoted here.

## THE MECHANISM OF INJURY

The mechanism of frost resistance cannot be understood without some knowledge of how frost injury is produced. We therefore discuss the latter first.

Müller-Thurgau in 1880 and many later authors have shown that to most plants of temperate and cool zones supercooling is harmless, even though it extends to temperatures far below that which is lethal when freezing occurs. (Injury to plants of warm regions by mere chilling is a different problem from that with which we are here concerned.) Frost injury proper therefore depends on ice formation. The usual locus of ice in plants frozen under natural conditions has long been known to be *outside* the cells, in the intercellular spaces. The size of the crystals decreases with the rate of freezing. With slow freezing large masses of ice may form in pockets produced by separation of cells. As the individual ice masses are often many times the size of all the adjacent cells, it follows that during freezing water may travel a considerable distance through the tissue. All the cells are more or less shrunken and collapsed in proportion to their loss of water to the ice crystals.

It has also been established that artificially rapid cooling of small sections, hairs and the like may induce ice to form *inside* the cells. Some authors, e.g. Schaffnit (1910), describe a condition termed frost plasmolysis, with a layer of ice between the protoplast and the wall—a position which is in a sense extracellular. It is possible that this condition is not produced by the actual freezing, since protoplasmic contraction (pseudo-plasmolysis) has frequently been observed in cells cooled to above 0° C. 'Vacuole contraction' might also be mistaken for plasmolysis. More commonly no plasmolysis is recorded, the ice being truly intracellular. Recently it was reported independently by Stuckey & Curtis (1938) and Siminovitch & Scarth (1938) that intracellular ice appears first in the cytoplasm and soon afterwards in the vacuole.

The visible effects of frost injury as observed after thawing are most commonly reported to be a contracted and coagulated protoplast with frequently a frothy structure. Except for the degree of contraction this appearance is not very distinctive from what is produced by many other killing agents. However, Luyet & Grell (1936) found that the contents of frost-killed in contrast to heat-killed cells are sufficiently fluid to be stratified by ultra-centrifuging. As with other modes of injury the tonoplast is frequently the last to suffer (e.g. Chambers & Hale, 1932). According to Levitt the gross morphological changes noted above and also survival of the tonoplast occur only with extracellular freezing.

The exact moment of injury being difficult to observe directly has been the subject of much controversy. Several authors have shown that in some plants death is revealed by changes in colour or odour while the tissue is still frozen. Iljin (1933–4), on the other hand, observed that on thawing the cells were killed as a result of rapid deplasmolysis and rupture of the protoplast, as will be explained later. Negative results in correlating injury

with rate of thawing have led most authors to the conclusion that injury normally precedes thawing.

In absence of direct evidence there are many theories as to how frost kills. The theories may be divided into three main groups according as they ascribe injury to (*a*) ice pressure, (*b*) mechanical effects of dehydration of the cells, and (*c*) physico-chemical effects of dehydration (frost precipitation theories). As regards the first, though hardy cells are in fact more resistant to pressure than unhardy (Levitt & Siminovitch, 1940), it is doubtful if the ice crystals exert general pressure on the tissues. The cells shrink because they lose water, not because of external pressure. Indeed, the whole tissue usually shrinks on freezing, showing that the volume of intercellular space can more than accommodate the expansion of water as it changes to ice. The second set of theories postulates tearing of the protoplast or damage to the plasma membrane (Maximov) during contraction in freezing or later expansion in thawing, to which type of injury the protoplasm is the more susceptible because of its dehydration. As regards the frost-precipitation theories the coagulated state of the cell after frost killing is of course no proof that death is caused by coagulation, nor is the fact that the freezing of plant juices produces colloidal precipitation, because among other differences the proteins can be adsorbed on the ice crystals in the juice while they are separated from them by cell walls in the tissues. Evidence from hardening changes which might tend to prevent precipitation will be discussed later.

So as to extend direct observation of the effects of freezing and thawing at different rates, Siminovitch (1938) studied sections kept in a micro-cold stage with strict control of temperature and using inoculation with ice to start crystallization when desired. For comparison whole potted plants were also frozen in a large cold chamber. Within limits it was possible to induce supercooling, extracellular freezing and intracellular freezing at will. These three phenomena occur in the order mentioned, but the succession may stop at the first or second step.

*Supercooling* alone never produced injury—which finding is in accordance with previous work.

*Intracellular freezing*. Though the first ice to form in sections is extracellular, intracellular freezing tends to follow not only when the temperature is lowered quickly but also when there is rapid crystallization on the breakdown of strong supercooling. Under the latter condition intracellular ice was observed even in whole plants kept in the refrigerator. Though in this case the rate of fall of temperature (from 0 to $-10°$ C. in half an hour) was high compared to what happens in nature (except perhaps in certain tissues when shade follows heating by the sun), this mode of ice formation may well occur in the open. Undercooling in nature to $-10$ or $-12°$ C. is not uncommon and has been reported to $-20$ and even $-32°$ C. It is possible that during a period of frost the ice may disappear from the cells again owing to the tendency of the larger crystals (which are outside the cells) to grow at the expense of smaller ones. This might explain why intracellular ice has rarely been observed in nature.

Intracellular freezing is nearly always fatal. Levitt found recovery in hardy cells of cabbage, but only if the amount of ice is slight. Even cells which can endure extremely low temperatures as long as ice is entirely extracellular are injured by a light frost if ice forms within them. According to Luyet & Thoennes (1938*a*), glassy ice (produced by extremely rapid cooling to below $-20°$ C.) is not injurious if the tissue can be thawed again without crystallization occurring. In ordinary intracellular freezing small crystals

ramify through the protoplasm, and the effect of these crystals in disorganizing essential structure and perhaps in causing surface precipitation might explain the peculiarly damaging effect of internal ice.

*Extracellular freezing.* This is the only type that is compatible with survival of any great degree of frost. As ice crystals grow in the intercellular spaces water is drawn from the cells and they collapse, wall and all, without plasmolysis. The degree of dehydration and shrinkage of the cells varies with the fall of temperature and, as long as they remain alive, it is reversed as the temperature rises again while still below the freezing-point of the sap at its normal volume.

Different modes and moments of injury were observed with this type of freezing as follows:

(1) *Critical temperature.* For any particular type and condition of cell there is a critical freezing temperature which is necessarily fatal. Correlation of our own and other results supports the view that the critical temperature of a tissue is correlated with its ability to endure dehydration by other means than frost (Scarth, 1941). Generally speaking, the lower the relative humidity of atmosphere and the higher osmotic pressure of solution a cell withstands the lower the temperature it can endure. Also drought hardening involves the same physiological changes as cold hardening.

In view of these resemblances and owing to the difficulty of observing and micro-manipulating cells in tissue which is either frozen or dried by evaporation, a study of the physical state of the protoplasm at various degrees of dehydration was made on plasmolysed cells (Levitt & Siminovitch, 1940–1). Viscosity increases with water loss as might be expected. The important point is that when a certain degree of plasmolysis is reached, a degree which depends on the frost and drought resistance of the cell, an irreversible stiffening (coagulation) occurs in a portion of the protoplast. Then, no matter how gradually the plasmolysing solution is diluted, deplasmolysis cannot proceed to completion before the protoplast ruptures. It is noteworthy that the plasmolysed cell seems to remain alive or at least semi-permeable after the coagulation occurs until it is damaged mechanically. Whether or not cells dehydrated by frost become dead when the critical temperature is reached is a rather academic question, because they are at least doomed to disintegrate as soon as thawing takes place, if not before.

(2) *Time factor.* There is a good deal of evidence that frost death at the critical temperature is rapid, but that very slow injury may occur above this point. Levitt found that the duration of the exposure to freezing temperatures has no distinct effect from a few minutes up to many hours, but produces increasing injury over a period of days. In dehydration by other means and at higher temperatures a time factor in coagulation is quite apparent over a short period and probably it is simply slowed down by low temperatures.

(3) *Mechanical effects of freezing.* There are grounds for inferring that mechanical injury may sometimes occur as a result of freezing at temperatures above the critical minimum. In the case of plasmolysed cells the protoplasm or a portion of it (see later) acquires a high consistency before it reaches the stage of irreversible coagulation. In that state it is readily torn by stretching or by passing a micro-dissection needle through it. When dehydrated by extracellular freezing, or by drought, protoplasm and wall adhere and the whole cell becomes not only shrunken but distorted. Frequently the walls invaginate until they meet in the middle of the cell cavity, pinching through the cytoplasm.

It seems probable that such disruption of protoplasmic structure may not always be repaired even when water is reabsorbed very gradually. We have no direct proof of cells being killed in this way, but from the susceptibility to mechanical injury of strongly plasmolysed cells and from the fact that cells (e.g. of trees) which have to endure very low temperatures are largely protected against such deformation, we infer that mechanical injury during fluctuations of temperature is a danger that has to be guarded against if cells are to endure very severe frost. Several authors have found that damage increased with successive freezings, notably in the case of trees frozen each time to just above their critical temperature (Day & Peace, 1937).

(4) *Thawing.* If the cells of sections have not been killed by freezing, survival is not always assured but depends in some measure on the rate of thawing provided the rise of temperature is large as well as rapid. Rapid thawing after a light freeze is without effect. Whether in nature injury ever results from a rapid thaw is still a moot point. Probably the only possible occasion is when the sun suddenly strikes frozen tissue. A sufficiently gradual rise of temperature (as happens when air temperature is the controlling factor) allows diffusion of water molecules from ice to cell sap while the temperature is still below the melting-point of ice, so that there is no flooding of intercellular spaces with water at any stage. The result is different when water is released faster than it can be absorbed. The cell wall is more permeable than the protoplasm so that it expands, first leaving the pseudo-plasmolysed protoplast behind. If not injured by this sudden tearing away of the wall the protoplast is liable to rupture in the violent deplasmolysis which follows as it too takes up water (Iljin, 1933; Siminovitch and Scarth, 1938).

(5) *Post-thawing period.* That damage may increase for some time after thawing has been reported by several authors, but it is not clear from these experiments whether the cells died because of previous frost injury to themselves or because of dead tissue around them. Levitt (1941) found that organs and tissues may survive both freezing and thawing yet die later unless given special treatment, viz. bathing in salt or sugar solutions of suitable osmotic pressure. Such cells have no doubt suffered frost damage which can be repaired under these special conditions. Low turgor pressure is perhaps indicated as the favourable factor, but more study of this phenomenon is needed.

## Modes and moments of injury

In brief, there appear to be three main types of injury due respectively to (1) intracellular freezing, (2) the mechanical effects of freezing and thawing when ice is extracellular, and (3) the physico-chemical effect of dehydration. The first occurs at the moment of rapid freezing of the tissue. The second is inferred to occur during fluctuations of temperature and is actually observed on rapid thawing. The third occurs at the critical low temperature, which marks the limit of frost endurance of the cell. It appears to be the commonest cause of death. How it acts will be discussed later.

## The mechanism of resistance

The mechanism of resistance is sought by first discovering what other characters are associated with hardiness and then inferring and if possible observing if and how they oppose the action of frost. Since there are several modes of injury we may expect to find several resistance factors but may also hope to discriminate the more important ones.

Certain phases of development of the plant and its cells, especially those of maturity and dormancy, are very commonly correlated with resistance. But though active growth in higher plants at least is not associated with great resistance, some degree of resistance and growth are not incompatible, while true dormancy is often unaccompanied by any resistance, although it would seem that any correlation between a particular phase of development and hardiness depends on the morphological and physiological characters of the cell which are commonly but not necessarily associated with that phase.

The one morphological character which seems to bear a relation to resistance is cell size. Very hardy plants or tissues have small cells, but such cells of course deharden without change in size so that the size factor can be of little importance unless associated with others.

Physico-chemical characters of the cell sap proved to be correlated with frost resistance are moisture content, sap concentration and sugar content, which form an interrelated group. Commonly the increase in sap concentration in hardening is partly due to water loss and partly and usually chiefly due to increase in total sugar. These factors might reasonably be expected to afford some protection, but as their correlation with resistance often breaks down other, more fundamental, ones must be looked for.

Study of the colloidal contents of the cell has been directed principally toward discovering whether hydrophilic quality goes along with hardiness. On the whole the evidence is against any necessary association of resistance with high pentosans, pectins, soluble proteins or other soluble nitrogenous substances. Several authors from Müller-Thurgau (1882) onward have reported an increase in the more soluble forms of nitrogen in plants exposed to hardening temperature, but the increase is not related to varietal differences in hardiness (Newton, 1924) and occurs also in tender plants that are injured by these temperatures (Wilhelm, 1935, 1935*a*; Dexter, 1935).

There is also no evidence in the literature that fats play any essential role.

Comparison of metabolic activity (e.g. respiration, photosynthesis and specific enzyme action) has generally shown that differences which tend to increase or conserve sugar promote hardiness but has thrown no further light on the problem.

Protoplasmic properties have only lately become the subject of direct experiment in this connexion. From 1931 onward (see Levitt, 1941) a number of Russian investigators have all agreed that cell permeability decreases with hardening. They estimated permeability from exosmosis, absorption of salts or turgidity. Exosmosis after freezing, as in the earlier work of Pantanelli (1919), was probably a measure of injury rather than of resistance and the other differences observed do not necessarily depend on permeability. We are convinced that the relation of permeability to hardening is the opposite of that stated above.

A comparison of the physical state of protoplasm in hardy and unhardy cells was first made by Kessler (1935) and extended by Kessler & Ruhland (1938). They found an increase in viscosity with hardening. While we would qualify this conclusion most of their results are not irreconcilable with our own described later.

From 1936 onward the McGill workers have published a series of cell physiological studies of hardening changes and the role they play. The phenomena investigated include osmotic pressure, bound water, permeability and the physical state of protoplasm including different regions of the protoplast. A summary of the results follows.

### Osmotic pressure

An increase in osmotic pressure, mainly due as a rule to conversion of starch to sugar, has long been recognized as a common accompaniment of hardening. The change is greatest in the hardiest types of cells. For example, it is over 400% in cortical cells of Caragana as measured plasmolytically and the difference would be still greater if measured cryoscopically, since the former method reduces the difference in water content. The two principal theories of the role of sugars in resistance are (1) reducing water loss by virtue of increased osmotic pressure which would also reduce mechanical strain, and (2) protecting the protoplasm from coagulation, since it has some protective action on proteins in exposed sap (Newton & Brown, 1931). But without other changes increase of sugar and osmotic pressure adds little to frost resistance. In fact, Levitt found that the rise in osmotic pressure to the winter maximum in tree cells occurred a week or two before any appreciable increase in hardiness. At most, therefore, this factor can serve only as an auxiliary to more important ones. Conditions such as lack of light or defoliation which prevent reserves from accumulating are often found to reduce hardening capacity in plants, but it is not proved that these act simply by preventing sugar concentration.

### Non-solvent space

This is a term applied to that fraction of the cell volume that does not behave as an ideal solution. It includes non-aqueous material and also bound or non-solvent water. As calculated it is $x$ in the formula $p(v-x)=$ a constant (within limits noted below), $p$ being osmotic pressure and $v$ cell volume. Its amount in herbaceous plants is usually too small to be detected by cell-volume measurements, but in tree cells it is high and rises in winter, reaching half the volume of the cell in *Catalpa*, for example. The increase here is due, in part, to lower water content. In extreme cases, as in seeds and spores, low water content causes non-solvent space to be the whole volume of the cell. A high non-solvent space representing high colloidal content has a much greater effect than high osmotic pressure in reducing or preventing ice formation and in minimizing shrinkage and deformation of the cell by frost. But in many cases resistance cannot be fully explained by these factors combined. For example, *Catalpa* in the unhardy state may have non-solvent space amounting to one-third of the cell volume, yet it is less frost resistant than hardy cabbage which has no appreciable non-solvent space and a rather lower osmotic pressure than the *Catalpa*.

### Bound water content

The term 'bound water' has been applied to water which remains unfrozen at some arbitrary temperature and alternatively to water which does not appear to act as solvent. The latter is also termed 'non-osmotically bound water'. Non-frozen water includes both osmotically and non-osmotically bound water. As we have dealt with osmotic relations already we need consider now only the non-osmotically bound fraction. There has been much search for a colloidal mechanism for binding water in hardy cells which might oppose the dehydrating effect of frost. As already stated, comparison of the colloidal material obtained from hardened and unhardened plants has revealed no certain increase of the more hydrophilic colloids as a feature of hardening. In most cases the amount of colloidal material is too small to have much effect on the cell volume. When, as in tree

cells, non-solvent space is large it is possible to obtain plasmometrically a rough estimate of the fraction of it which is non-solvent water. Since bound water varies with total water its amount in the cell will vary with the degree of plasmolysis, and in proportion as $x$ in the expression $p(v-x)$ is bound water it too will vary. Estimates based on this relationship indicate that tree cells contain significant amounts of bound water which are maximal in winter and fall to a minimum in spring (Levitt & Scarth, 1936). As regards herbaceous plants in which the colloidal content of the cell is low, Levitt's measurements (1939) on cabbage, using a combination of calorimetry and cryoscopy, may be quoted as indicating the condition. The non-osmotically bound water is so small as to be within the experimental error, but according to the actual figures it is somewhat higher in the hardened plants. At the respective critical temperatures, however, it is reduced to a much lower level in the hardy than in the unhardy, and without doubt the total bound (unfrozen) water is about $4\frac{1}{2}$ times greater in the non-hardy. This means that the non-hardy cell is reduced to half its normal volume and the hardy to less than one-third at the respective death-points.

It is evident from all these results that frost resistance is not due simply to prevention of dehydration of the whole cell or, as far as the evidence goes, of its colloids. However, few if any of the bound water results give sufficient information regarding the condition in the living protoplasm. Most of the estimations were made on dead tissue or extracts, and in any case the critical difference in hydrophily (if it exists) may reside in a small fraction of the protoplasm. It is noteworthy that *Catalpa* cells show an enormous increase in the thickness of the cytoplasmic layer on hardening which might be due to increased imbibitional force. Others (e.g. apple) show no change, but nevertheless there may be increased swelling pressure to balance the increased osmotic pressure of the vacuole. There will be occasion to revert to this question.

## CELL PERMEABILITY

Levitt & Scarth (1936) demonstrated clearly, and others have confirmed, that an increase in cell permeability to water and other polar substances accompanies hardening. The more hardy the cells the greater the subsequent increase, and tree cells are many times more permeable in winter than in summer. There is also no doubt that rapid loss of water from cells affords protection against the danger of intracellular freezing. Experiments with the micro-stage show that this type of freezing is more difficult to produce in hardy than unhardy material, and that it develops first in those cells of a preparation which, owing to their distance from ice crystals, lose water more slowly. The more rapidly water leaves the cell in consequence of extracellular freezing the sooner does the cell solution concentrate till its freezing-point drops to the actual temperature and the less chance is there of it freezing too.

## PROTOPLASMIC VISCOSITY

A comparison of the viscosity of hardy and unhardy protoplasm is difficult for several reasons. (1) Apparent viscosity or consistency in a substance like protoplasm has two components, true viscosity and structural viscosity, which may vary oppositely. In some cases a discrepancy between the Brownian movement test on the one hand and micromanipulation and deplasmolysis injury (which varies with viscosity) tests on the other, suggests that true viscosity is higher and structural viscosity lower in the hardy cells. (2) The physical state of cytoplasm is not uniform throughout its thickness as explained

later. (3) Differences in specific gravity and starch content render centrifuge tests unreliable. (4) Difference in osmotic pressure complicates the comparison especially in tests involving plasmolysis.

Kessler & Ruhland's (1938) findings showed a higher viscosity in hardy cells. Levitt & Siminovitch (1940, 1941) obtained divergent results with different methods on cells which were not plasmolysed (or otherwise dehydrated), but on the whole they agree that Kessler & Ruhland's general conclusion is likely, especially as regards true viscosity. On the other hand, when both kinds of cells are dehydrated by an equal force the condition is definitely reversed whatever test is used. Structural viscosity predominates in proportion as the cells are dehydrated, and it is this type of consistency which seems to be responsible for the greater susceptibility to mechanical injury of unhardy as compared with hardy cells in plasmolysing media of the same osmotic pressure which corresponds to equal freezing temperature.

## Coagulation

At a certain stage of dehydration, as already stated, the stiffening of the protoplasm becomes irreversible, a condition customarily termed 'coagulation'. Differences between cells as regards the osmotic pressure of the solution in which they undergo this change is perhaps a truer index of their relative resistance to low temperature than any other criterion except the direct test of freezing. This is true of different species as well as the same species in different states of resistance.

## The cell surface

We have obtained evidence from various modes of micro-manipulation that plant cells resemble certain animal cells in the differentiation of their cytoplasm into several concentric zones. On the outside there seems to be an extremely thin and relatively fluid *surface film* (which reveals itself by spreading over an applied oil drop and lowering its surface tension). Beneath this is a more definitely proved narrow zone of relatively high structural viscosity, the *ectoplasm* or *plasmagel layer*. The ectoplasm overlies a broader zone of fluid, *endoplasm*, *mesoplasm* or *plasmasol region*, which in vacuolated cells is bordered on the inside by the tonoplast (Scarth, 1942).

The effects of dehydration already mentioned, namely, increased viscosity and ultimately coagulation of the protoplasm, appear first and are most apparent in the layer of ectoplasm, and it is here that the difference between hardy and unhardy protoplasm is perhaps greatest. But the surface film too shows striking differences. It has a much lower refractive index in hardy cells—apparent also in the relative visibility of the Hechtian strands that stretch between a plasmolysed protoplast and the cell wall. These strands also show far greater ductility in hardy cells. For example, they snap and crumple up in non-hardy *Hydrangea* in 3 *M* dextrose, while in hardy they may remain fluid and often flow together in 6 *M*. Further indication of colloidal change at the cell surface as a result of hardening is the previously noted increase in permeability of the plasma membrane (Siminovitch & Levitt, 1941; Scarth, Levitt & Siminovitch, 1940).

## Correlation of protoplasmic properties with hardiness

The importance that can be attached to the protoplasmic changes as to others will depend on how widespread is their correlation with hardiness. It may be noted that the direct effects of non-injurious low temperature on the permeability and consistency of proto-

plasm whether hardy or unhardy are the reverse of the indirect effects which only develop during hardening. In all comparisons of hardy and unhardy cells both were kept at the same temperature.

Scores of plants of varied type have been tested for permeability change during hardening and dehardening. It has invariably been found in plants which harden and never in those which do not. The rise is greatest in plants which develop a high resistance and seems to vary with the degree of resistance. Whether the variation is quantitatively proportional even in the same cell at different times is doubtful, but no such close correlation is to be expected for one of many factors.

As regards the physical state of protoplasm the only tests that have been applied to a wide range of plants are plasmolysis and deplasmolysis injury. Increased toleration of plasmolysis and increased survival of rapid deplasmolysis after non-lethal plasmolysis invariably accompanied hardening. Resistance to both is greatest in very hardy cells and is roughly parallel to frost (and also drought) resistance among different varieties of wheat (Whiteside). Compared with this good correlation for the protoplasmic changes, osmotic pressure and hardiness frequently varied independently within the same selection of plants.

## PHYSICO-CHEMICAL NATURE OF HARDENING CHANGES

As we have seen, the key factors in resistance are protoplasmic. The others reinforce the protoplasmic but by themselves are ineffective. Most important of all, because it protects against the commonest type of injury and determines the minimum temperature the plant can withstand, is the reduced tendency to coagulation of the ectoplasm.

There is no evidence that coagulation is prevented in any great degree by an increase in protective substances such as sugars or a decrease in *p*H or electrolyte concentration. The change would seem to be in the labile colloids themselves. The next step in the study of mechanism is to explain this difference in terms of colloid chemistry.

Recently, Siminovitch (working now at the University of Minnesota, initially with the guidance of the late Dr Gortner) has begun a study of this difficult problem. His immediate objective is to discover chemical or physico-chemical differences in the proteins (undenatured if possible) and fats comparing inner bark of trees hardened and unhardened. So far the only marked difference he has found is in soluble proteins—soluble between *p*H 5 and 7. There is at least twice as much in the hardy and it amounts to at least half the total protein extracted. The quantity of insoluble protein and total lipids seems to be the same in both. It is suggestive that not only resistance to coagulation but all the other protoplasmic hardening changes, namely, reduced structural viscosity (with perhaps higher true viscosity), increased permeability, lower refractive index and in some cases increased bound water, could conceivably be explained by the kind of colloidal change which the analytical difference implies, that is, increased hydrophilic quality. However, coagulation of protoplasm, if it is anything like coagulation of blood, may be a complex process involving lipids as well as proteins, and permeability too is supposed to be regulated by lipids. Accordingly, further study of qualitative changes within the lipid and lipo-protein groups is indicated and is still in progress.

## Summary of resistance

The three main types of frost injury mentioned earlier are opposed by the various hardening changes as follows:

(1) Intracellular freezing tends to be prevented by increased cell permeability to water because this accelerates concentration of the cell sap by the growth of ice outside the cells.

(2) Mechanical injury during freezing and thawing with ice extracellular is principally prevented by the reduced 'structural viscosity' of the cytoplasm, or, at least, of its outer zones. Hardy cytoplasm preserves a more fluid or ductile consistency than unhardy when exposed to equal dehydrating force. The comparison was made on plasmolysed cells but is presumed to hold for cells dehydrated by frost. Further protection is usually afforded by increased osmotic pressure and in very hardy cells by high non-solvent space, both of which reduce shrinkage and distortion of the cells.

(3) Dehydration injury at the critical low temperature is prevented by reduced coagulability of the protoplasm—again notably of its ectoplasm.

(4) The protein fraction which is soluble between *p*H 5 and 7 is twice as great in Robinia bark in summer as in winter.

(5) Increased hydrophily of cytoplasmic proteins indicated by this chemical difference, could conceivably account for all the protoplasmic hardening changes.

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# A NOTE ON THE THERMODYNAMIC ASPECT OF RESPIRATION

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This brief note is intended to draw attention to one thermodynamic aspect of respiration following upon the paper of Wohl & James (1942).

Respiration is the fundamental activity of all living tissue, serving as a source of energy and of chemical intermediates for synthetic processes. Wohl & James have shown that synthesis utilizes only a small part of the energy liberated in respiration. It is usual to regard the excess energy liberated as heat as waste and a sign of inefficiency. It is the purpose of this note to point out that the heat liberated is the end-product of a number of separate reactions, and that it is one manifestation of the energy which has determined the orderly progression of the respiratory process.

In the higher plants carbohydrates are the main respiratory substrate although protein metabolism is probably linked with carbohydrate catabolism. The mechanism of respiration consists of a large number of linked reactions, the product of each forming the substrate of its successor. These reactions are either reversible or irreversible. This distinction may be arbitrary in that irreversibility may be the reflection of energy relations of such magnitude that the reaction does not appear to proceed at all in one direction, or the absence of a mechanism whereby sufficient energy can be applied to drive a reaction in the direction necessary to establish the reversibility of the system. Of the reactions, some will proceed spontaneously, these reactions resulting in a reduction of free energy in the system, others must be driven in the required direction by added energy. Since more energy is liberated by spontaneous reactions than required by the driven ones, the substrate breaks down at a steady rate.

The reactions of respiration are divisible into two groups, those which are hydrolytic and those which are oxidative. The latter group is the principal source of energy. These oxidation reactions form a step-by-step series whereby, according to the viewpoint taken, hydrogen or electrons are transferred between the respiratory substrate and oxygen. It is frequently stated that the oxidation systems form a graded series of increasing potential up to oxygen. This is only correct if the electron transfer is the same in all the systems. The factor controlling the position of any one system in the oxidation series is the free-energy change which results from that particular reaction. The relationship of the free-energy change to the potential for any reversible reaction involving electron exchange is established in (1).

$$-\Delta G = nFE, \tag{1}$$

where $E$ is the e.m.f. and $nF$ represents the number of coulombs which must pass for the reaction to proceed to the extent represented by the chemical equation. $-\Delta G$ is the free-energy change. The free-energy change is related to the operating conditions in (2):

$$-\Delta G = RT\ln K_c - RT\Sigma\nu\ln c, \tag{2}$$

where $K_c$ is the equilibrium constant in terms of the concentration of the reactants. The term $RT\Sigma\nu\ln c$ is comparable to $RT\ln K_c$ but it involves the concentrations of the experiment and not those at equilibrium, $\Sigma\nu\ln c$ is the algebraic sum of all the $\nu\ln c$ terms and $\nu$ is the number of mols of the reactants involved, the resultants are taken as positive and the reactants as negative.

Combining (1) and (2)

$$-\Delta G = RT\ln K_c - RT\Sigma\nu\ln c = nFE.$$

The potential of any one of the reversible redox systems in the respiratory process is proportional to the free-energy change and is related to the concentrations of the reactants and the products in the tissue and those at the equilibrium point of the reaction.

The free-energy change is as much a characteristic of the reaction as are the chemical substances involved. It will determine the position of the reaction in the respiratory scheme, since it will fix what other reaction it may drive or by what other reaction it may be driven according to the direction of the free-energy change. It may also influence the rate at which the whole process of respiration moves. An illustration of this point may be secured from a study of the polyphenol oxidase system. The oxidase is capable of oxidizing any compound containing the catechol unit and certain monohydroxy phenols. A redox system is formed with the corresponding quinone possessing a standard potential whose value is determined by the structure of the molecule of which the phenol is part. When such substances are added to respiring tissue they vary in their effect on the respiration rate as measured by the $O_2$ uptake and the $CO_2$ output. It may be that the difference between the effect of one substance and that of another on the rate of respiration is due to the difference between the potentials of their systems.

The difference between the effects of anaerobic conditions on the respiration rates of sliced and whole potato tubers is most striking: on transferring washed slices to anaerobic conditions respiration is irreversibly inhibited after about 180 min., whereas the whole tuber will respire anaerobically at a subaerobic rate for some days and within certain time limits, will, when returned to aerobic conditions, respire at approximately the previous level. It would appear improbable that in the slices some respiratory intermediate accumulates so rapidly and in such quantity that in so short a time it is toxic to the tissue, but that this substance does not so accumulate in the whole tuber under anaerobic conditions. The explanation of this difference may be found in the kinetic aspect of respiration. In the whole tuber the concentrations of respiratory intermediates are such that the changes which result from the transfer to anaerobic conditions do not disturb the free-energy values of the reactions sufficiently over the space of a few days to bring about the disintegration of the mechanism of respiration. However, as the result of the slicing of the tuber, certain modifications occur in the respiratory mechanism. When this tissue is transferred to anaerobic conditions, then the changes which occur in the concentrations of the respiratory intermediates are sufficient to modify the free-energy relations of the integrated reactions of respiration to such an extent that a rapid and irreversible destruction of the mechanism occurs.

The heat liberated in respiration is the final manifestation of the free-energy changes of a large number of separate reactions. These individual changes maintain the orderly progress of the respiration mechanism.

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# ON THE VARIABILITY OF THE DESMID *XANTHIDIUM SUBHASTIFERUM* WEST

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(With 4 figures in the text)

Since the early days of systematic investigation many authors have treated the subject of variability in the desmids. There are two main reasons for the large literature on this subject, one being the high degree of differentiation of the cells, providing many easily observed morphological details, the other the special mode of division typical of the desmid cell. This mode of division theoretically preserves any morphological change for an unlimited period. The desmid cell, consisting of two semicells, divides in such a way that at every cell division one new semicell is formed by each half of the mother cell. Thus each cell is made up of two halves which may differ in age of origin from one to an indefinite number of cell generations. So far as is known at present, the two halves from an original single cell, if they survive, should remain unchanged after an indefinite number of divisions. Again, assuming the survival of all the daughter cells, the mode of division of the desmid cell results in the retention and propagation of every variation that may arise. This might well account for the very large number of variations reported without in any way necessitating a higher rate of occurrence of such variations in desmids than in any other group.

Until recently field collections were the only source of study, and conclusions of necessity were only tentative. The need for cultures was stressed by several authors, for instance by Ducellier (1915), but technical difficulties were too great until culture methods were worked out in detail. Pringsheim (1918) outlined the principles for the successful cultivation of desmids, which he followed (1930) by a study on the growth of two *Micrasterias* species, in which he demonstrated that under 'favourable conditions', i.e. as long as the division rate was high, the typical form only was produced, but with a medium giving a lower division rate a mixture of types appeared leading eventually to degenerate forms. Czurda (1926–35) in his extensive work on Zygnemales came to very similar conclusions and introduced the terms 'progressive' and 'regressive' phase in a culture or assembly of cells. Only the progressive phase is of real importance for comparative work and for a definition of the type. In 1936 Ondraček published the first comprehensive study on variability in desmids under controlled conditions. The species he chose, however, show very little differentiation and only some aspects of variability could be studied. Reference will be made later to his conclusions. Lefevre (1939) gave a very interesting account of variability of desmids in culture and an extensive bibliography is appended.

The present report on observations of *Xanthidium* is of a preliminary nature. While working with various culture media, to test their effect on the division rate of plankton organisms, it was found that *Xanthidium* responded very readily to changes of conditions. The results were striking in that a number of cell types could be produced which were

intermediate between *X. subhastiferum* and some of its described varieties and between this species and *X. antilopaeum* (Bréb.) Kütz., as well as other combinations of characters not present in known species. These cells, for the first time produced in culture, apparently bear the same relation to the known species of *Xanthidium* as do the many aberrant forms, found in the field and illustrated widely in the literature, of other genera to the typical forms of those genera. Ducellier (1915) gave a good summary of the relevant literature upon aberrant forms in different desmid genera, added a number of new observations from his own collections, and developed the theory which has now been proved by direct observation, namely, that variations may occur when the new semicell is affected by changed conditions during its formation, while the old semicell retains its original structure. Bennett's suggestion (1889) that an intermediate form of *Euastrum* had arisen by hybridization appears extremely improbable in the light of recent work. Only cultures including the sexual cycle and germination of zygospores could bring convincing proof of such a claim, and it may be taken for granted that Bennett's form arose in much the same way as the intermediate forms here under discussion. The theoretical

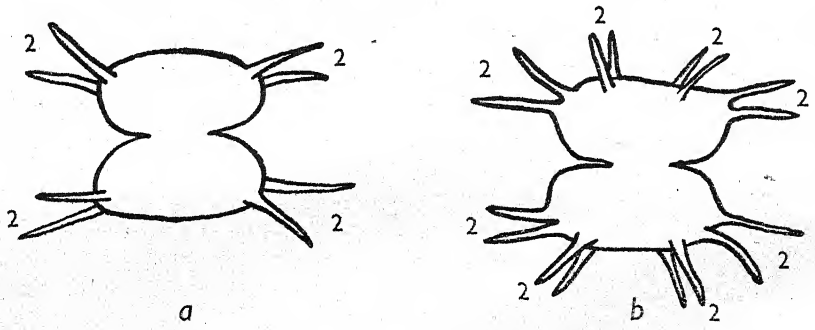


Fig. 1. Diagrams of two types of *Xanthidium* cells with the formulæ (*a*) 22/22, (*b*) 2222/2222.

possibility of a preceding hybridization cannot logically be excluded, but may be discarded as highly improbable. So far no case of hybridization among the desmids has been reported.

In presenting the results it has been found convenient to give formulae for the different cell types rather than diagrams. As shown in Fig. 1, those points where spines arise are provided with a figure indicating the number of spines. The first figure refers in all cases to the lowest spines on the left upper side and the succeeding figures are given in a clockwise order moving round both semicells. In cases where there are no spines produced in any particular position a 0 is given. The number of figures also indicates the shape of the semicell, two figures implying only two points, four figures four points of spine formation. The formulae for the two semicells are separated from one another by an oblique stroke, the formulae for Fig. 1 *a* being thus: 22/22, and for Fig. 1 *b* 2222/2222. In some cases no normal spines were formed, but only stumps or warts, and these are indicated in the formulae by *w* following the figure which indicates their number.

In an appendix the chemical composition of the media is given. Glass-distilled water was used throughout and for all liquid cultures pyrex dishes were employed. All cultures were kept at a north window. The temperature was not controlled and fluctuated between 13 and 15°.C.

The cell material used was isolated from net plankton catches from the North Basin of Windermere, English Lake District. The initial material was collected in March before the diatom maximum, when the water was relatively rich in nutritive salts (see Rosenberg, in the press). The water temperature was below 10° C. All cells collected and used for experiments had the constitution 22/22.

The first divisions of isolated cells in the laboratory gave rise to 22/2112 cells in the medium N/2 50. Fig. 2 (*a*) is a camera lucida drawing of this type, showing the well developed spines in the new semicell. Subsequent divisions in clone Xa 25 then gave rise to normally developed 2112/2112 cells (Fig. 2*b*). This unexpected result led to further experiments with a view to determining the factors responsible for spine formation.

In 15 clones changes in cell shapes and structure were studied under varied conditions. It soon became clear, however, from the multiplicity of types which arose, that for any exact physiological analysis controlled experimental conditions would be

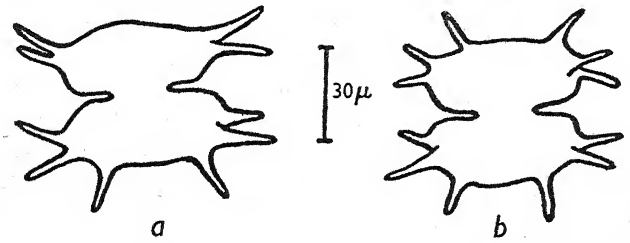


Fig. 2. *Xanthidium subhastiferum*, two cells developed in cultures.

Table 1

| Types of cells | N/2 50 10 cultures | Soil extract 4 cultures | Agar 10 cultures |
|---|---|---|---|
| Spines normal | 1 | 1 | 0 |
| Spines increased | 4 | 0 | 0 |
| Spines normal and increased | 1 | 0 | 0 |
| Spines reduced | 3 | 2 | 8 |
| Spines increased and reduced | 0 | 0 | 2 |
| Spines normal and increased and reduced | 1 | 1 | 0 |

essential. These controlled conditions have not been available, and the studies have been correspondingly limited.

In all cases division rate under available conditions was low, generally one division in 5 days. The low light intensity was thought to be mainly responsible for the long intervals between successive divisions.

Cell shape, size and spine development were the factors the variation of which was observed; in most cases the history of an individual cell was followed.

Among liquid media employed only N/2 50 and soil extract will be considered, and among solid media only agar made up with Benecke solution N.

Table 1 gives a summary of conditions observed and shows that very complex reactions took place. The fact that apparently opposed reactions such as spine increase and reduction may take place in the same medium at the same time implies either differences in localized conditions at the moment of division or determination by the past history of the cell. It is more likely from various observations mentioned later that the latter factor is

operative. Parallel with the change in number of spines the shape of the semicells varies, becoming angular with an increase, and becoming rounded with a reduction, in number of spines. The size of the semicells is reduced with a reduction of spine number, and is much smaller than in semicells of the original type taken from old cultures with a low division rate.

In the liquid medium N/2 50 the increase in number of spines is of special interest. While a number of 2222/2222 cells were found in cultures arising from 22/22 cells, the increase under special observation was limited to 2112 or 2012 types in the new semicells. This difference occurred in parallel cultures isolated on the same date, and may be due to individual cell variations, the original material not being sufficiently homogeneous. In future work this factor could be excluded by continued cultivation in bulk under standard conditions before individual cells are tested for their reactions. The clone Xa 30 was remarkable in that one cell pair produced new semicells which were mirror images, i.e. the same combination of spines arose at the point of contact, thus: 22/2012 and 2102/22.

This observation, which was confirmed in the medium N/2 25, provides a possibility of induced spine formation, one semicell affecting the other at some early stage of development. Nothing is, of course, known at the present stage about the physiological details of such development.

As an example of the high number of different types which can arise from 22/22 cells the clone Xa U is of interest. An examination on 3 August of the cell material produced in N/2 50 from a transfer of 22/22 cells into fresh medium on 20 July (i.e. after 14 days) gave the following result. Twelve types of cells were recorded in the following proportions:

| | |
|---|---|
| 2 00/00 | 3 22/12 |
| 4 00/11 | 3 22/22 |
| 7 22/00 | 1 22/2012 |
| 1 22/20 | 1 22/2112 |
| 4 22/11 | 1 02/2101 |
| | 1 2112/2112 |
| | 1 2211/2112 |

From a total of twenty-nine cells all except three show, after division, a change in spine formation in the new semicells. Twenty-three of the fifty-eight semicells of all ages present in the culture show the original structure 22, fifteen are spineless, eleven show a reduction from 2 to 1 spine (eight on both insertion points and three on only one insertion point), two show an entire loss of spines at one insertion point, and seven have four insertion points compared with the two of the original type. One cell, 2112/2112, has reached the stage where both semicells are the same, and provided conditions remain unchanged, this structure should continue. This cell type, as well as the 00/00 in which again both semicells are the same, is of particular interest, as both correspond directly to other species or even genera. If the history of the cells were unknown a 00/00 type would certainly be described as a *Cosmarium* sp. and the 2112/2112 either as a variety of *Xanthidium subhastiferum*, for instance var. *Johnsonii* (W. & G. S. West) G. M. Smith, or even as a variety of *X. antilopaeum* (Bréb.) Kütz.

The intermediate stage has been superseded by a balanced stage, showing symmetry about the transverse axis, separating the semicells, as well as about the longitudinal axis. As mentioned before, cells were observed which showed symmetry about the transverse axis only, and in which the left halves of both semicells were similar but different from the right halves. An example is the type 2102/2012 produced in cultures, which may well remain stable or develop into 2112/2112. It is impossible to say from the available data

whether or not these cells indicate the induction of morphological characters in the new semicell by the old semicell. Such may be the case, although the semicells have hitherto been considered as more or less independent units.

The other medium used, soil extract, gives roughly the same variety of types but shows a marked difference in grouping. Eleven cell types were recorded from a total of twenty-nine cells. The types of the fifty-eight semicells are summarized in Table 2 and compared

Table 2

| Type | Number of semicells produced | |
|---|---|---|
| | N/2 50 | Soil extract |
| 22 | 23 | 27 |
| 11 | 8 | 1 |
| 00 | 15 | 5 |
| Quadrangular | 7 | 20 |
| Odd | 5 | 5 |
| Total | 58 | 58 |

with the results for N/2 50 medium. The frequency of the type 00 is only one-third of that found in N/2 50, while the quadrangular semicells are almost three times more frequent. The quadrangular semicells are of the type 2112 in nine cases, and 2102 or 2012 in eleven cases. Only one balanced quadrangular cell was found in this culture, viz. 2102/2012, which is symmetrical about both axes. It is impossible at the moment to develop any physiological theory as to the cause of this different reaction in the two media if it is significant. Soil extract, a highly complicated solution, appears on the evidence available to contain substances of an unknown nature which favour the formation of spines.

On agar spine formation is generally reduced to wart formation, while the shape of the new semicells is in the great majority of cases quadrangular. Fig. 3 shows the result of two successive divisions of an individual 22/22 cell on the agar surface. This medium is ideal for the observation of single cells and their history, but since warts form instead of normal spines, chemical or physical factors seem to be operating to increase the number of insertion points, hence favouring quadrangular semicells, whilst at the same time reducing the length of the spines to warts. An example is the division of the cell 22/22 resulting in 22/2*w* 0 1*w* 2*w* and 22/2*w* 2*w*. In another example of successive divisions on agar, the cell 2*w* 2*w*/1*w* 1*w* 1*w* 1*w* gave 2*w* 2*w*/1*w* 1*w* 1*w* 1*w* and 1*w* 1*w* 1*w* 1*w*/2*w* 1*w* 1*w* 2*w*. Entirely rounded semicells are also produced; a case such as 22/2*w* 1*w* 1*w* 2*w* giving 22/2*w* 2*w* 2*w* 0 and 2*w* 1*w* 1*w* 2*w*/0000 is typical. Fig. 4 shows a *Cosmarium* type produced on agar in the clone Xa 1b. The measurements of this cell are 46·2 $\mu$ long and 36·3 and 33 $\mu$ for the widths of the two semicells as compared with the measurements for the 22/22 type 54 $\mu$ long, 55–56 $\mu$ wide, showing a considerable reduction in size typical for the 00/00 cells.

It appears from the results so far obtained that variations in cultures of *Xanthidium subhastiferum* affect (*a*) the points of spine insertion and hence the cell shape and (*b*) the length of spines produced. It seems clear that this distinction is artificial, only chosen for convenience of description, for when the length of the spine reaches zero the result will be a rounded semicell just as when the number of points of insertion is reduced to zero. It appears reasonable, however, to work on the assumption of the existence of these two

trends in modifications of the cells of *Xanthidium*, producing cells of very different systematic standing. Future work will have to attempt an analysis of the causes of the ob-

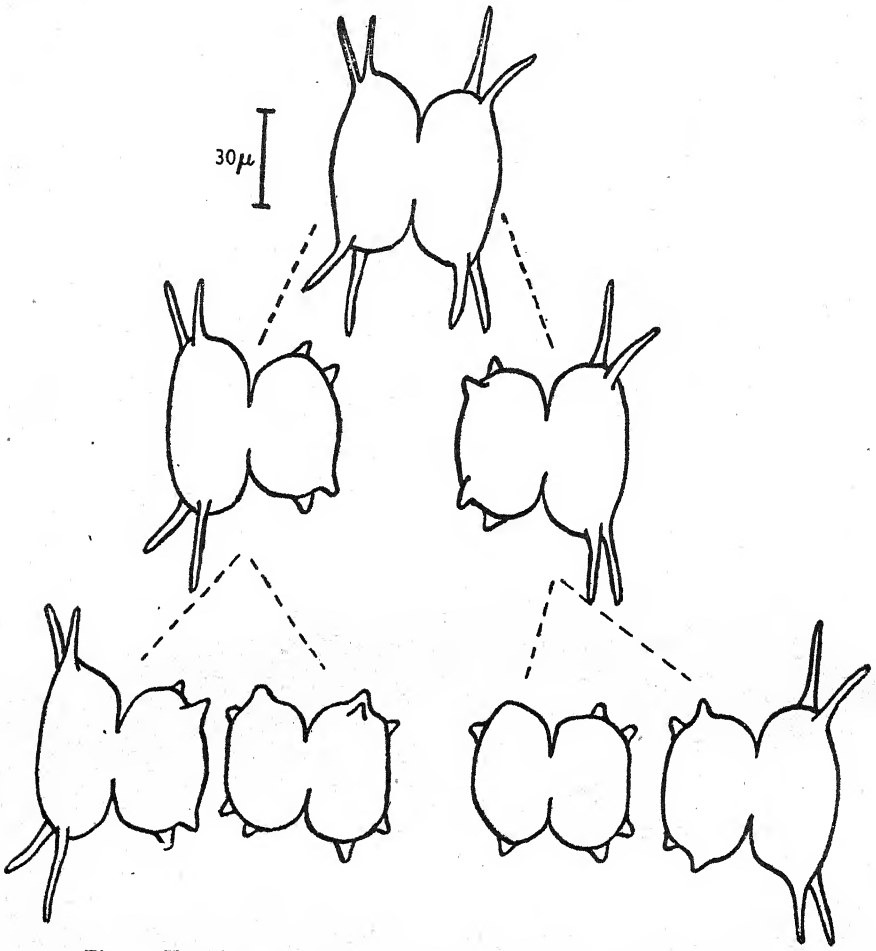


Fig. 3. *Xanthidium subhastiferum.* Cell types arising from two successive divisions starting with a 22/22 cell, on agar.

served variations as they are of considerable importance for the systematic aspects of morphology.

In addition to laboratory material plankton samples were examined for *Xanthidium* cells. The samples were routine horizontal tows from the North Basin of Windermere. Since this method of sampling bears no definite relation to the volume filtered no quantitative results could be obtained, but only relative proportions of the species found. At least 200 cells or colonies were counted from each sample. When these results were sifted for the type of *Xanthidium* cells present, varying totals were obtained. Any statistically sound counts would have to be based on the same totals for each date. Table 3 is therefore only of interest as to the cell types observed and gives but slight indication of a possible shift of types during the period covered by the samples. The intermediate types en-

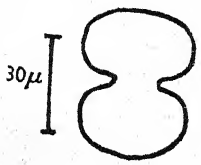


Fig. 4. *Xanthidium subhastiferum.* A 00/00 cell produced on agar.

countered were 22/2012, 22/2112 in May, 12/21, 2112/2102 in June, 2112/2222 in August, 22/2102, 22/2112 in December.

Reynolds (1940) reports another case of seasonal variation of two varieties in *Staurastrum paradoxum*. A form with two processes is more abundant in autumn, winter and spring, while the common type with three processes is dominant in midsummer. Intermediate forms are more common at a time of change-over of dominance. This appears to be the only record of a more detailed study of the relative frequencies of varieties. It is suggested that the variety with three may arise from that with two processes, but the reverse process is not held to occur. In the light of observations on *Xanthidium* it seems very likely that both an increase and a reduction of processes may occur, given the right conditions.

A detailed discussion of the relevant literature, which is very extensive, is not intended here. The papers by Jacobsen (1875), Klebs (1879), Schmidle (1893), G. S. West (1899), de Wildeman (1895), Playfair (1910), and West, W. and G. S. (1904–23), cover the knowledge obtained from field collections, and embody a fair amount of theoretical speculation. In many instances the need for cultures is emphasized. Ondraček's paper (1936) has the most immediate bearing on the present observations. In agreement with other workers he

Table 3. *Types of cell in plankton tows*

| | Number observed | | | |
|---|---|---|---|---|
| | 22/22 | Intermediate | 2222/2222 | *w* |
| 28 May | 7 | 4 | 0 | — |
| 6 June | 6 | 2 | 0 | — |
| 29 June | 2 | 1 | 0 | — |
| 6 July | 4 | 0 | 0 | — |
| 27 July | 41 | 0 | 0 | 1 |
| 3 Aug. | 39 | 1 | 1 | — |
| 10 Aug. | 7 | 0 | 1 | — |
| 18 Aug. | 18 | 0 | 2 | — |
| 13 Sept. | 13 | 0 | 3 | — |
| 7 Dec. | 27 | 8 | 1 | — |

comes to the conclusion that homogeneous cell material can be maintained if the division rate is high: with a drop in frequency of cell divisions the proportion of aberrant cells rises. This latter part of his conclusions can be confirmed for *Xanthidium*. It is certainly desirable to test whether the new cell types can be maintained, and this seems very likely under standard conditions.

The implications for systematics from such work are that the diagnosis of *X. subhastiferum* should be extended to cover forms found in the cultures described here. It is not intended and not thought to be desirable to add a number of varieties to those already existing. On the contrary, it may be expected that further work will reduce this number considerably, since it has already been shown that the cell types which can arise are by no means stable genetically fixed types, but only phenotypic modifications. Future work in desmid taxonomy will have to make more use of the method of culture, particularly in comparison of different lines to determine what genetical predispositions they may severally have to certain kinds of modification. It must be remembered that since conditions in nature very rarely approach and hardly ever maintain for a long time 'the optimum' for cell division, so we should expect to find considerable variability in field collections. Similar conclusions have been reached by most workers on the experimental morphology of algae since Chodat, and will no doubt affect the development of all algal taxonomy.

SUMMARY

The variability of the desmid *Xanthidium subhastiferum* West was studied in clones in various media. A considerable range of variation was observed in the number and length of spines and in the number of their points of insertion, the latter variations resulting in a change of shape of the semicell. Soil extract favoured the production of spines, while the inorganic medium used reduced spine formation. The histories of several extreme types of cells were followed and intermediate stages of their formation were recorded. Without a knowledge of their history several of the types found would be classified as different species or even genera from that of the mother cell. Plankton samples were examined for different cell types and a considerable number of intermediate forms was found. The implications for desmid taxonomy are discussed.

## APPENDIX

### *Composition of the media used*

Benecke solution, used with 1–2 % agar, referred to as Be N

| | |
|---|---|
| $NH_4NO_3$ | 200 p.p.m. |
| $CaCl_2$ | 100 p.p.m. |
| $K_2HPO_4$ | 100 p.p.m. |
| $MgSO_4$ | 100 p.p.m. |

N/2 50 used as liquid medium only

| | |
|---|---|
| $NH_4NO_3$ | 50 p.p.m. |
| $CaCl_2$ | 50 p.p.m. |
| $K_2HPO_4$ | 50 p.p.m. |
| $MgSO_4$ | 50 p.p.m. |

Soil extract after Pringsheim (1936).

Equal weights of garden soil and water are boiled for 1 hr., allowed to settle, and the supernatant liquid decanted. This extract is diluted 1 : 10 with distilled water.

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# STUDIES IN *VIOLA*

## IV. THE SOMATIC CYTOLOGY AND TAXONOMY OF OUR BRITISH SPECIES OF THE GENUS *VIOLA*

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(With 18 figures in the text)

CONTENTS



### 1. Introduction

It is apparent now that cytology can be of great use in taxonomy, for much of cytological research is purely morphological, dealing with the numbers, sizes and shapes of the chromosomes. As Turrill (1938) puts it, morphological cytology enables the systematist to advance from an alpha taxonomy towards an omega taxonomy. Chromosome numbers alone can often be of use in determining the position of species within a general classification, but sometimes they have only a limited applicability. For instance, several morphologically distinct species may have the same chromosome number, just as several morphologically indistinguishable plants may have different chromosome numbers. A complete description of the karyotype would in cases like these simplify the problems involved, but it is only possible to work out a complete picture of the somatic chromosome complement in some few cases.

There is still considerable confusion as to the exact systematic position of our British species of *Viola*. The present paper attempts to give an analysis of the chromosome complements of some of them in relation to their classification. Some new numbers are given and an attempt has been made to determine each complement by measurements of chromosome lengths. All methods of measuring chromosome lengths are open to considerable criticism—the error must be relatively great. Granted this, however, the results need not necessarily be vitiated. If, for example, the chromosomes of a set fall into three or four obvious and clear-cut size classes, such as small, medium and large, it does not matter whether the *exact* measurements of those of any individual class be made, providing that the amount of error does not lead to confusion of the chromosomes of any one class with those of another class. Thus a 'formula' can be obtained, which, added to the number and general description of the chromosomes, gives one more morphological characteristic for the aid of the systematist. This 'group method', as it can be called, of classifying chromosomes has already been successfully applied to the classification of the genus *Urtica* (Fothergill, 1936).

All somatic counts and drawings were obtained from root-tip material fixed in Langlet's modification of Nawaschin's fixative and stained in aqueous gentian violet according to the technique described by the author in the first paper of this series. Only suitable chromosome plates in complete cells were chosen for drawings from which measurements were to be made. These were drawn with an Abbé camera lucida at a magnification of about 4050. Several chromosome plates were drawn as necessary, and the chromosomes were measured with a pair of very fine dividers.

## 2. CHROMOSOME NUMBERS AND DESCRIPTIONS, ETC.

As the drawings and the analyses of the chromosome lengths in Table 1 show, the chromosomes of these species of *Viola* tend to fall into four length groups, namely *A*, *B*, *C* and *D*. Group *A* represents the relatively very long chromosomes characteristic of *V. tricolor* and its varieties; they also occur in *V. nana*. In some of the other species relatively long chromosomes were present, but these were obviously not similar to those of *tricolor*, hence they were designated *B* (long). The *C* chromosomes are those with a medium length; they showed a great range in actual length, but it was found to be impracticable to subdivide the group further. Practically all of the species possessed a few very small chromosomes falling into the *D* class. The numbers in each class then give a formula, and the validity of its use in systematics rests on the assumption that the chromosomes of any one set will contract at equal rates during mitosis. At a magnification of 4050, 1 mm. equals 0·246 $\mu$.

Table 1. *Analysis of chromosome lengths, and length classes*

*Note.* All the varieties of a collective species like *V. tricolor* have not been included in this table.

| Species | Chromosome number $2n$ | Length classes in centimetres | | | | | | | | Basic formula (haploid) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Class *A* | No. in *A* | Class *B* | No. in *B* | Class *C* | No. in *C* | Class *D* | No. in *D* | |
| *V. lutea* | 48 | — | — | 12·0–12·5 | 2 | 5·0–10·5 | 46 | — | — | 1*B*, 23*C* |
| *V. nana* | 48 | 14·0–14·5 | 2 | — | — | 4·0–11·0 | 46 | — | — | 1*A*, 23*C* |
| *V. ruralis* | 34 | — | — | 11·0–12·0 | 2 | 6·0–9·0 | 30 | 3·5 | 2 | 1*B*, 15*C*, 1*D* |
| *V. Curtisii* | 26 | 14·0–16·0 | 4 | — | — | 8·0–12·5 | 20 | 6·5 | 2 | 2*A*, 10*C*, 1*D* |
| *V. variata* | 26 | 15·0–18·0 | 4 | — | — | 8·0–11·5 | 20 | 6·0 | 2 | 2*A*, 10*C*, 1*D* |
| *V. sulphurea* | 28 | 11·0–14·0 | 5 | — | — | 6·0–9·5 | 21 | 4·0 | 2 | (2*A*, 10*C*, 1*D*)+(*A*, *C*) |
| *V. Pesneaui* | 26 | 14·5–15·5 | 4 | — | — | 6·5–13·5 | 20 | 4·5–5·0 | 2 | 2*A*, 10*C*, 1*D* |
| *V. canina* | 40 | — | — | — | — | 4·5–8·0 | 38 | 3·0 | 2 | 19*C*, 1*D* |
| *V. Riviniana* | 40 | — | — | — | — | 4·5–7·5 | 38 | 3·0 | 2 | 19*C*, 1*D* |
| *V. contempta* | 40 | — | — | — | — | 4·5–7·5 | 38 | 3·0 | 2 | 19*C*, 1*D* |
| *V. Silvestris* | 20 | — | — | 9·0 | 2 | 4·5–6·5 | 18 | — | — | *B*, 9*C* |
| *V. hirta* | 20 | — | — | — | — | 5·0–8·0 | 18 | 3·0–3·5 | 2 | 9*C*, 1*D* |
| *V. odorata* | 20 | — | — | 9·5–10·0 | 2 | 5·0–8·0 | 18 | — | — | *B*, 9*C* |

*Sect. Nominium, subsect. Uncinatae*

*V. hirta* L. My material of this species came from Snowshill, East Gloucester. The chromosome number is $2n=20$ (also Clausen, 1926, 1927; Gershoy, 1934; Heilborn, 1926). The formula is 2 (9*C*, *D*). The chromosomes are medium sized and many possess constrictions.

*V. odorata* L. This was an unusual specimen of very small habit and with a tendency towards pubescent peduncles. It is a native plant of unknown origin, and its varietal name is unknown. It came from East Meon, South Hants.

Fig. 1. *Viola variata*, $2n=26$.

Fig. 2. *V. variata* var. *sulphurea*, $2n=26+2$.

Fig. 3. *V. Curtisii*, first meiotic metaphase, polar view showing 13 bivalents.

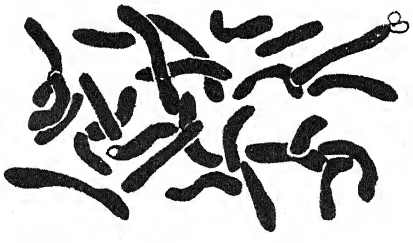

Fig. 4. *V. Curtisii*, $2n=26$.

Fig. 5. *V. Pesneaui*, $2n=26$.

Fig. 6. *V. Lloydii*, $2n=26$.

All somatic chromosomes from root-tips unless otherwise stated. Magnification ×4050.

Fig. 7. *Viola odorata*, $2n=20$.

Fig. 8. *V. hirta*, $2n=20$.

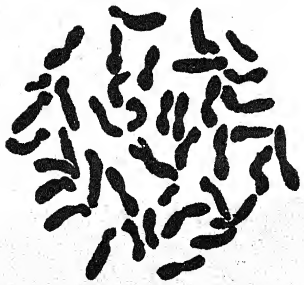

Fig. 9. *V. Riviniana*, $2n=40$.

Fig. 10. *V. Silvestris* (= *V. Reichenbachiana* Bor.), $2n=20$.

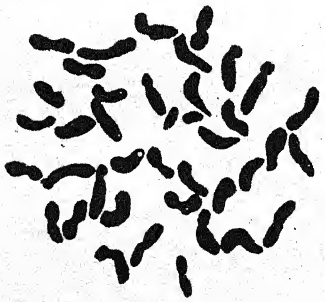

Fig. 11. *V. canina*, $2n=40$.

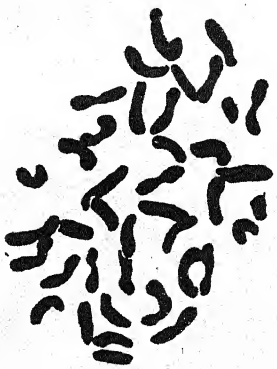

Fig. 12. *V. contempta*, $2n=40$.

All somatic chromosomes from root-tips unless otherwise stated. Magnification $\times 4050$.

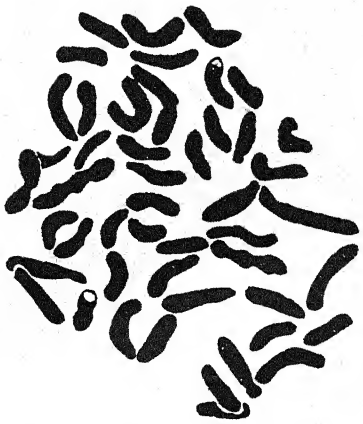

Fig. 13. *V. lutea* var. *amoena*, $2n=48$.

Fig. 14. *V. nana*, $2n=48$.

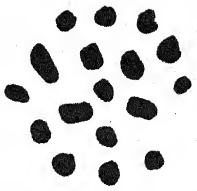

Fig. 15. *V. Deseglisei*, first meiotic metaphase, polar view with 17 bivalents.

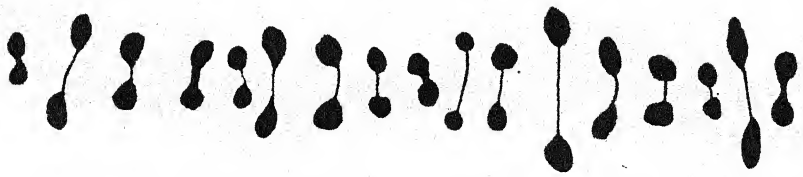

Fig. 16. *V. Deseglisei*, first meiotic metaphase with all the bivalents arranged side by side, showing the regular division.

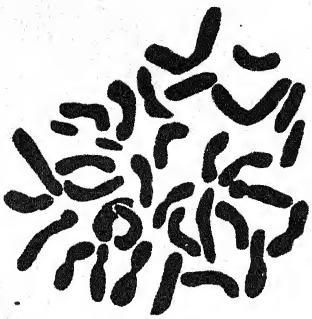

Fig. 17. *V. ruralis*, $2n=34$.

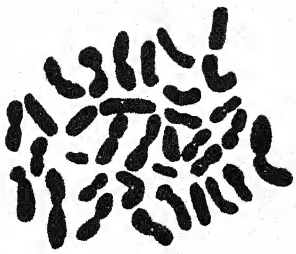

Fig. 18. *V. Deseglisei*, $2n=34$.

All somatic chromosomes from root-tips unless otherwise stated. Magnification $\times 4050$.

*V. odorata* L. var. *subcornea* (Jord.) Parl. from Bullington, North Hants. This is merely a colour variety of native origin.

*V. odorata* L. var. *semperflorens* Hart. = var. *praecox* Greg. is an autumn-flowering form, probably of garden origin (from P. M. Hall, garden).

*V. odorata* L. var. *immaculata* Greg. corresponds to the garden variety 'White Cran'. It came from St Mary Church, South Devon, and is of naturalized origin.

*V. odorata* L. from Welcombe, Warwickshire, varietal name unknown.

All these varieties of *V. odorata* L. have $2n=20$ chromosomes (also Clausen, 1926, 1927; Heilborn, 1926; Miyaji, 1929, $n=10$; and Gershoy, 1928, $2n=18$; later, 1934, $2n=20$). All of them have the common formula 2 (*B*, 9*C*). The chromosomes are very similar to those of *V. hirta*, and some possess median, others subterminal constrictions. The chief difference between the two sets is that *odorata* possesses a pair of long *B* chromosomes which are absent in *hirta*, while the latter has a pair of *D* chromosomes absent in the former. This difference is constant, and enables one to distinguish between a chromosome complement of one or the other.

### *Subsect.* 2. *Rostratae*

*V. canina* L. specimens came from Craster, Northumberland; Brown Down, South Hants, growing on damp shingle near the sea; Allerthorpe, Yorks; and Otmoor, Oxfordshire.

*V. Riviniana* Reichb. from Gibside, Durham.

The chromosome number of these two species is $2n=40$ (also Clausen, 1926, 1927, $n=36$; Brunn, 1932, $n=20$; Gershoy, 1934, $2n=40$). From visual analysis the chromosome sets are almost identical, and the formula for both is 2 (19*C*, *D*). Many of the chromosomes are medianly or subterminally constricted.

*V. Silvestris* Lam. (= *V. Reichenbachiana* Bor.) from Lower Bullington, North Hampshire, has $2n=20$ chromosomes (also Clausen, 1927, $n=10$; Gershoy, 1928, $2n=42$, later, 1934, $2n=20$). The formula is 2 (*B*, 9*C*). The chromosomes seem to resemble those of *canina* and *Riviniana*, but unlike these two species *Silvestris* possesses a pair of *D* type chromosomes.

### *Sect. Melanium, subsect. Tricolores*

(*a*) Kitaibeliana *series*

*V. nana* Corbière, from the Botanical Gardens, Potterne. Chromosome number $2n=48$, and formula 2 (1*A*, 23*C*).

(*b*) Tricolor *series*

All of these species possess 26 somatic chromosomes with the formula 2 (2*A*, 10*C*, *D*). They include the following: *V. variata* Jord. from Lintzgreen, Durham; *V. Lloydii* Jord. from Isle of Raasay, Hebrides, and from Prestwick Carr and Wooler, Northumberland; *V. Lejeunei* Lloyd & Foucaud from the Botanical Gardens, Potterne; *V. segetalis* Jord. from Kirkley, Durham; *V. Curtisii* Forst. from St Cyrus, Aberdeen and Llanmadoc, Gower Peninsula, South Wales (also $n=13$); *V. variata* var. *sulphurea* from Wooler, Northumberland. The specimen of this yellow-flowered, tricolor type showed $2n=28$. On analysis the formula of 2 (2*A*, 10*C*, *D*) + (*A*, *C*) showed that this was merely a variant

with one extra *A* and one extra *C* chromosome. My colleague, Dr K. B. Blackburn, has found other specimens to possess 26 chromosomes.

All these numbers of the *tricolor* series of pansies are new.

*V. tricolor* L. has long been known to have the somatic number $2n=26$, or the meiotic number $n=13$ (see Clausen, 1921, 1922, 1931 *a*, *b*; Fothergill, 1938; Gershoy gave $2n=24$ for some varieties). The chromosome sets of this series are distinctly different from those of any of the other series examined because of the possession of four very long chromosomes. Many of them are medianly, or subterminally, constricted.

*V. contempta* Jord. from the Botanical Gardens, Potterne, on several occasions. The chromosome number is $2n=40$, and the formula is 2 (19*C*, *D*).

(*c*) Arvensis *series*

This series here includes *V. ruralis* Boreau, sent as *V. arvensis* from the Botanic Gardens, Cluj; *V. Deseglisei* Jord. from Stakeford, Northumberland and Beacon Hill, Northumberland (also $n=17$); and *V. obtusifolia* Jord. from Northumberland (also $n=17$). The common chromosome number is $2n=34$, and the numbers are new, although *V. arvensis* Murr. has previously been given as $n=17$ (Clausen, 1921, 1922). The common formula is 2 (*B*, 15*C*, *D*). In appearance the sets of chromosomes seem to be intermediate between those of *V. lutea* and *V. canina*, and to bear little resemblance to those of the *tricolor* series.

(*d*) Lutea *series*

*V. lutea* Huds. var. *amoena* Hens. from Devil's Elbow, Scotland, has $2n=48$ chromosomes (also Clausen, 1931, $n=24$; Gershoy, 1934; Fothergill, 1938; and Griesinger, 1937, $2n=38$–40). The formula is 2 (*B*, 23*C*). The *lutea* chromosomes grade into one another as far as their lengths are concerned, making it difficult to analyse them, but the complement does possess one large pair of *B*-type chromosomes. Median and subterminal constrictions are present.

*V. nana* Corbière from the Botanical Gardens, Potterne, has $2n=48$ and a formula of 2 (*A*, 23*C*). The complement is very similar to that of *lutea* with the difference that it possesses a pair of very long *A*-type chromosomes and no *B*-type ones.

## 3. Classification, discussion and conclusions

The genus *Viola* is a large one with over three hundred species, and many of the chromosome numbers are known, giving the following series: $n=6$, 10, 12, 13, 17, 20, 27, 30, 36, and 48. Miyaji (1929) finds that the *Chaemelanium* and *Dischidium* sections of the genus fall into a polyploid series with $x=6$ as the basic number, while the *Nominium* section has a base number of $x=10$. On this he builds up a suggested scheme of relationship. This is possibly too simple a solution of the problems of the affinities of the members of the genus, as a glance at the above list will show. While polyploidy has probably played a part in the evolution of the genus, it has not been the only factor at work. *Violas* hybridize very readily—wild hybrid populations are by no means uncommon in this country—while Clausen (1931 *b*) and Gershoy (1928) have produced many hybrids. Gershoy lists 161 of these produced from parents of all degrees of affinity True, only those between very

closely related species were fertile: the wide crosses between various types were all sterile. This may not be so in nature, for Fothergill (1938) has described a pure breeding hybrid type from a wild hybrid population between *V. lutea* and *V. tricolor*.

(*a*) *The* Nominium *section of the genus*

From chromosome number and morphological considerations the *Nominium* violets, *V. hirta* L. and *V. odorata* L., are closely related; so also are *V. canina* L., *V. Riviniana* Reichb., and *V. Silvestris* Lam. W. Becker (1910) in the *Viola Europeae* and in Engler and Prantl's *Natürlichen Pflanzenfamilien* divides the *Nominium* violets as follows:

Sectio Nominium Ging.
A. Rostellatae Boiss.
1. Scapigerae W. Bckr. ined.—Pl. Acaules.
(*a*) Curvata-pedunculata W. Bckr.
(α) Flagellatae Kittel.
*V. odorata.*
(β) Eflagellatae Kittel.
*V. hirta.*
(*b*) Erecto-pedunculata W. Bckr.
2. Axilliflorae W. Bckr. ined.—Caulescentes.
(*a*) Mirabilis Nym.
(*b*) Rosulantes Borb.
*V. Riviniana, V. Silvestris.*
(*c*) Arosulatae.
(α) Caninae W. Bckr.
*V. canina.*
(β) Arborescentes.
B. Plagiostigma Godr.

Rouy & Foucaud in their *Flore de France* (1896) divide the section as under:

Section 1. Nominium.
Subsection 1. Rostellatae Boiss.
Group 1. Caulescentes Nob.
(*a*) includes *V. canina* L.
(*b*) includes *V. Silvestris* and one of its two 'formes'.
*V. Riviniana* Reichb.
Group 2. Acaules Nob.
includes *V. hirta* L. and *V. odorata* L.

The cytological formulae indicate that *V. hirta* and *V. odorata* are rather different in their chromosome constitution, the one being 2 (9*C*, *D*) and the other 2 (*B*, 9*C*), which supports the usual separation of these two species into two different series of Becker's group, the *Curvata-pedunculata*. All the varieties of *odorata* are cytologically identical. The classification above separates *canina* from *Riviniana* and *Silvestris*. The last two are placed by Becker in his group *Rosulantes* of the series *Axilliflorae*, while Rouy & Foucaud consider *Riviniana* to be a 'forme' of *Silvestris*. Their cytology certainly supports the view that they are closely related, as shown by their inclusion in the larger group *Axilliflorae* or *Caulescentes*. It also indicates quite as clearly that their final position may require

some revision. *Canina* and *Riviniana* are almost indistinguishable in their chromosome complements, and their common formula is 2 (19*C*, *D*). Neither of them, however, seems to be a simple polyploid from *Silvestris*, or a *Silvestris* type, for this species possesses a pair of long *B*-type chromosomes and lacks a pair of *D*-type chromosomes. Both *canina* and *Riviniana* could have arisen from a *Silvestris* type by doubling of the whole set of chromosomes and fragmentation of this *B*-type chromosome. This chromosome seems to possess a subterminal constriction, and the length of the small distal portion is about the same as the length of the small chromosomes of both *canina* and *Riviniana*.

*V. canina* requires special detailed consideration. This species was found by Clausen (1931*c*, 1933) to be a type with an oscillating chromosome number—he considered it to be a species recently formed and in process of establishing itself. This is unlikely, as *V. canina* has such a wide geographical range. Originally, Clausen (1927) published the number $n=36$ for this species. This count was based on examination of pollen mother cells of a type of the species obtained from Virum, near Lyngby (Sjaelland, Denmark). The divisions were apparently quite regular. Later, however, plants from the same clone showed a somatic chromosome number of 47, not 72. Further investigations of flower buds showed that in side views of the first meiotic metaphase nearly all of the chromosomes functioned as univalents, but behaved as bivalents in that they arranged themselves in the equatorial region and showed constrictions. In polar view, this gave an impression that more bivalents were present than was really the case.

In other types from different localities Clausen found irregular meiotic divisions. For instance, from Bidstrup, the variety *lucorum* shows 17 univalents scattered all over the spindle, giving rise to different numbers at the poles. Another type, from Hornbeck, and another from Ljungby, showed dwarf pollen in the anthers, indicating irregularity. The most regular type came from Tisvilde and showed 20 bivalents plus a number of fragments.

Clausen concluded that *V. canina* is a cytologically irregular species maintaining itself through constant intercrossing between different types belonging to the species. He also invited co-operation with other cytologists. Brunn (1932) responded to this invitation and used material of *canina* which grew at Rickömberg, near Uppsala, Sweden. He examined root tips from about 38 different plants, and in each case he found the chromosome number to be $2n=40$, with no fragments, or extra chromosomes, present. Brunn then entered upon speculations as to the origin of the variations in Clausen's Danish material. Later, however, Clausen (1933) pointed out that Brunn overlooked a prime difference in their material—Clausen's types, with one exception, came from uncultivated habitats occupied by native vegetation, whereas Brunn's material came from a habitat which was influenced by cultivation. Miyaji (1929), on theoretical grounds, doubts the validity of Clausen's count of $n=36$ for this species, for the *Nominium* violets mostly have 10 as the basic number. The plants of *V. canina* examined for this paper grew in wild habitats and came from four widely scattered areas, and in all cases the chromosome number was undoubtedly $2n=40$. Furthermore, the complement of chromosomes agrees with that of a near relative, *V. Riviniana*. The chromosome number of *canina* is thus brought into line with that of the rest of the violets of the *Nominium-Rostellatae* section of the genus. From Clausen's descriptions of the cytology of his types it would seem that they were of hybrid origin. The behaviour of the chromosomes at meiosis closely resembles the behaviour of the chromosomes of a wild hybrid population between *V. tricolor* and *V. lutea* (Fothergill, 1938).

Drabble in his *Notes on the British Pansies* (1927*a*) includes *V. contempta* among the *tricolor* series, and morphologically this seems to be its correct place in the classification. Its chromosome number, however, is $2n=40$, and the chromosome complement is much closer to *canina* than even *Silvestris*—the formula is exactly the same, viz. 2 (19*C*, *D*). The chromosomes themselves, as the respective figures show, are unlike those of any of the *tricolor* types. Rouy & Foucaud in their *Flore de France* (1896) put variety *θ contempta* (=*contempta* Jord.) in their collective species *V. saxatilis* Schmidt. Drabble takes objection to this on account of the perennial habit of *contempta*. In fact, Drabble makes these two characters important diagnostic ones separating series of species. But I have cultivated so-called annual pansies for three years. Perhaps the importance of this character has been over-emphasized from a diagnostic point of view.

*V. contempta* thus presents a problem. Unfortunately, the chromosome numbers of *V. lepida* and *V. carpatica*, the two remaining members of Drabble's *saxatilis* series, are not known. Neither are those of Rouy & Foucaud's series. The problem, therefore, requires further investigation before even a tentative suggestion as to the true relationships of this species can be given.

(*b*) *The* Melanium *section of the genus*

There is considerable difference of opinion as to the classification of the pansies. All the British pansies are included in the group *Tricolores* of W. Becker in Engler and Prantl's *Natürlichen Pflanzenfamilien*. (We have already seen the problem raised by *V. contempta* Jord.) *V. lutea*, however, is separated from the collective species *V. tricolor*. Rouy & Foucaud (1896) include it in their collective species. These latter systematists divide *V. tricolor* up as follows:

*Viola tricolor* L.
Forme: *V. hortensis* DC.
*V. saxatilis* Schmidt.
*V. arvensis* Murray.
β *Lloydii* Jord.
δ *Variata* Jord.
*V. Kitaibeliana* Roem. & Schultes.
subspec. *V. Curtisii* Forst.
*V. lutea* Huds.

By the word 'forme' is to be understood an aggregate of varieties where a variety is taken to mean something similar to a 'race' in horticulture. The varieties in a forme exhibit variations or modifications which may be due to change of habitat, and these are not of sufficient importance to warrant the 'forme' specific rank. The forme then is a group of similar varieties.

Drabble in *The British Pansies* (1926, 1927*a*,*b*,*c*, 1928) later classifies our British types into five series as below:

Series 1. *Arvenses*.
2. *Tricolores*.
3. *Saxatilis*.
4. *Luteae-Curtisii* (1927*c*).
5. *Nanae*.

Thus Drabble divides the forme *V. arvensis* Murr. of Rouy & Foucaud by taking from it the large-flowered species *V. variata*, *V. Lloydii*, and *V. Lejeunei*, and putting them in a new group, the *Tricolores*. Drabble's group *Arvensis* includes all the English small-flowered species. He also separates *V. lutea* and *V. Curtisii* from Rouy & Foucaud's forme *Kitaibeliana*, and along with *Pesneaui* he makes a new series of them. Becker, too, separates *lutea* from *tricolor*. The chromosome numbers and formulae undoubtedly support some of these separations. *V. arvensis* Murr. has $2n=34$ chromosomes and the formula 2 (*B*, 15*C*, *D*), while *Lloydii* and similar varieties possess 26 somatic chromosomes, with the formula 2 (2*A*, 10*C*, *D*). *V. nana*, the English type of *Kitaibeliana*, possesses 48 chromosomes resembling those of *lutea*, but unlike those of *Curtisii* which has $2n=26$.

Clausen criticizes Drabble's multiplicity of species on the ground that chromosome number can often be of value in taxonomy. Drabble's series the *Arvenses* includes *V. segetalis* Jord., *obtusifolia* Jord., *ruralis* Boreau, and *Deseglisei* Jord. These all possess 34 somatic chromosomes with the common formula 2 (*B*, 15*C*, *D*), and it has proved to be very difficult to distinguish between them cytologically. There is then no cytological reason why these four types should not be regarded simply as *V. arvensis*. Morphological differences between them are not clear cut, and each type is subject to considerable variation according to its environment. J. L. Crosby (1939) is of this opinion, and, although he finds that plants from one batch of seeds are more or less alike, he thinks the small differences between plants of different batches are due to slight changes in genic constitution. On the other hand P. M. Hall (1937) considers that *V. ruralis* Jord. is absolutely identical with *V. variata* var. *sulphurea* (except for the one character of flower size). The cytological findings here show that *ruralis* is definitely an *arvensis* pansy and not a *tricolor* one.

The series *Tricolores* includes *V. contempta* Jord., *V. Lloydii* Jord., *V. Lejeunei* Jord., *V. variata* Jord., and *V. variata* Jord. var. *sulphurea*. The cytology of these and other species does not entirely support this classification. *Lloydii* and *variata* possess the chromosome number of $2n=26$, and this is the number for *V. tricolor* L. These *tricolor* species have a complement of chromosomes which is easily distinguishable from that of the members of the other series or collective species by the possession of four large *A*-type chromosomes. The variety *sulphurea* which I examined possessed 28 chromosomes, but my colleague, Dr K. B. Blackburn, tells me that specimens from the same population examined by her possessed the expected number of 26 chromosomes. On analysis the 28-numbered forme shows the presence of 5*A*, 21*C*, and 2*D* type chromosomes, i.e. a formula of 2 (2*A*, 10*C*, *D*)+(*A*, *C*). The population from which it was taken was morphologically homogeneous, and so far as is known it is then genetically pure. Without further detailed cytological examination (now no longer possible) it seems probable that this plant arose through a method of somatic dislocation of some type. If this is the case then var. *sulphurea* falls into line with the rest of these 26-numbered pansies. *V. contempta* Jord. has already been dealt with.

Drabble originally separated the *Lutea-Curtisii* series into two distinct groups. The *Luteae* group included only *V. lutea* Huds. and its colour variety *amoena* Hens. Later, however, he came to consider that *Curtisii* Forst. and *Pesneaui* Lloyd & Foucaud were merely forms of *lutea* Huds. 'Some are colour forms, others are ecads' (1927*c*, p. 219). Both the *Curtisii* forms examined in this paper (the one from Llanmadoc, and the other

from Aberdeen) and *Pesneaui* have 26 somatic chromosomes, with the same formula as the *tricolor* pansies *variata* and *Lloydii*. Rouy & Foucaud (1896) consider *Curtisii* Forst. to be a subspecies of *tricolor* L. Thus it would seem that, on cytological grounds, both of these species will have to be removed from the *lutea* group, and put in the *tricolores* series. Incidentally, whichever group *Curtisii* does belong to, it is merely an ecological variety, dwarfed, and growing on sand dunes usually near the sea. On transference to a garden, or plant pot, the stems elongate and the whole plant comes to resemble a normal-sized yellow-flowered *tricolor* pansy.

Unfortunately no species of the *Saxatilis* series were examined. According to Drabble the group comprises only *V. lepida* Jord., and its forms like *V. carpatica* Borbas. Clausen (1927) states that the chromosome number is $n=13$.

Drabble's *Lutea-Curtisii* series then contains only *V. lutea* and its colour variety *amoena* Hens. *Lutea* is distinct from *tricolor* in both chromosome number ($2n=48$) and formula 2 (*B*, 23*C*). *V. nana* Corbière in its distribution reaches only as far as the Channel Islands. It seems to correspond to *γ nana* DC., a forme of *V. Kitaibeliana* Roem. & Schult. This plant (*V. nana*) is also an ecological variety of sand dunes and shingles. Drabble himself noted that in a different environment the plant enlarged and became Rouy & Foucaud's subvariety *major*. His findings were confirmed by Brother Louis Arsène of Jersey (Drabble, 1928, p. 160). In chromosome number and constitution of the chromosome set *V. nana* agrees closely with *V. lutea*. From the figures it will be seen that the sets are very much alike. Both possess 46 *C*-type chromosomes, while they differ in that *lutea* has 2 *B*-type ones which are lacking in *nana*, and the latter has 2 *A*-type ones which are lacking in the former. This close similarity indicates an affinity between these two species, and so perhaps supports Rouy & Foucaud's classification of the *Kitaibeliana* group of *Viola* species in which *V. nana* is a 'forme', and *V. lutea* a subspecies of *V. Kitaibeliana* Roem. & Schult.

## 4. SUMMARY

1. The research is concerned with the cyto-taxonomy of some of our British species of *Viola*. The value of cytology as a handmaiden to taxonomy is emphasized.

2. The following new chromosome numbers are given:

$2n=20$, the varieties *subcornea*, *semperflorens* and *immaculata* of *V. odorata* L.;

$2n=26$, *V. variata*, *V. Lloydii*, *V. Lejeunei*, *V. Pesneaui*, *V. segetalis*, *V. Curtisii*, and *V. variata* var. *sulphurea* (special case in which $2n=26+2$);

$2n=34$, *V. obtusifolia* (and $n=17$), *V. ruralis*, and *V. Deseglisei* (and $n=17$);

$2n=40$, *V. contempta*;

$2n=48$, *V. lutea* var. *amoena* Hens., *V. nana*.

3. The following numbers are confirmed: *V. odorata* L., *V. hirta* L., and *V. Silvestris* Lam. with $2n=20$; *V. canina* L. and *V. Riviniana* Reichb. with $2n=40$.

4. These species were obtained from a wide variety of localities.

5. The chromosome number of *V. canina* is considered in some detail.

6. By an analysis of the chromosome complements the somatic chromosomes were found to fall into four well-defined size groups: *A*, very long; *B*, long; *C*, medium; and *D*, small. Thus each species could be given a formula.

7. The application of the cytology of the group to their taxonomy has given rise to the following conclusions.

A. *The* Nominium *section of the genus.* *V. hirta* and *V. odorata* are closely related but their separation into two distinct groups by W. Becker is justified. Becker puts *V. Riviniana* and *V. Silvestris* into the same section of the *Rosulantes*, while Rouy & Foucaud consider that *Riviniana* is merely a 'forme' of *Silvestris* in the group *Caulescentes*. Cytologically there is as close, if not closer, affinity between *canina* and *Riviniana* than between either of these two and *Silvestris*. The chromosome number of *Silvestris* is half that of the other two, but these two are not just simple polyploids. Polyploidy plus fragmentation of a long *B*-type chromosome may account for their origin.

B. *The* Melanium *section of the genus.* The series *Tricolores*, *Luteae-Curtisii* and *Nanae* of Drabble require some revision from the cytological evidence. Cytologically *Lloydii*, *Lejeunei*, *variata*, *variata* var. *sulphurea*, *Pesneaui*, and *Curtisii* belong to the same group, the *Tricolores*. Thus *Pesneaui* and *Curtisii* are extracted from Drabble's *Luteae-Curtisii* group which is left with the single species *V. lutea* and its variety *amoena*. This separation of *lutea* from *tricolor* spec. coll. is justified, but *V. nana* and *V. lutea* are seen to have very similar chromosome complements. Drabble's creation of a separate series, *Nanae*, is not perhaps necessary, while the cytological evidence rather supports Rouy & Foucaud's inclusion of both *lutea* and *nana* in the collective species *V. Kitaibeliana* Roem. & Schult. Finally, *V. contempta* requires further investigation. In chromosome number, etc., it resembles the $2n=40$ series, while systematically it is placed among the *tricolor* pansies with $2n=26$.

I would like to express my thanks to my colleague, Dr K. B. Blackburn, and Mr P. M. Hall of Fareham, Hants, for so very kindly supplying me with abundant material, and for helping in the identification of the varieties.

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# STUDIES ON THE BRITISH VOLVOCALES

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(With 41 figures in the text)

## I. Two new volvocalean flagellates

In the course of examination of samples of fresh-water algae, several new species have been encountered: two such species, which came into my hands before the present war, were studied as completely as the material permitted; in neither was it possible to make a complete study of the life history. Brief mention has been made of both of these plants (Jane, 1938, 1939), but it was hoped to obtain further information before they were named and described in detail. As there appears to be scant prospect of obtaining further supplies of material in the near future, it seems desirable that the information available should be placed on record, to be supplemented later, should further details become known.

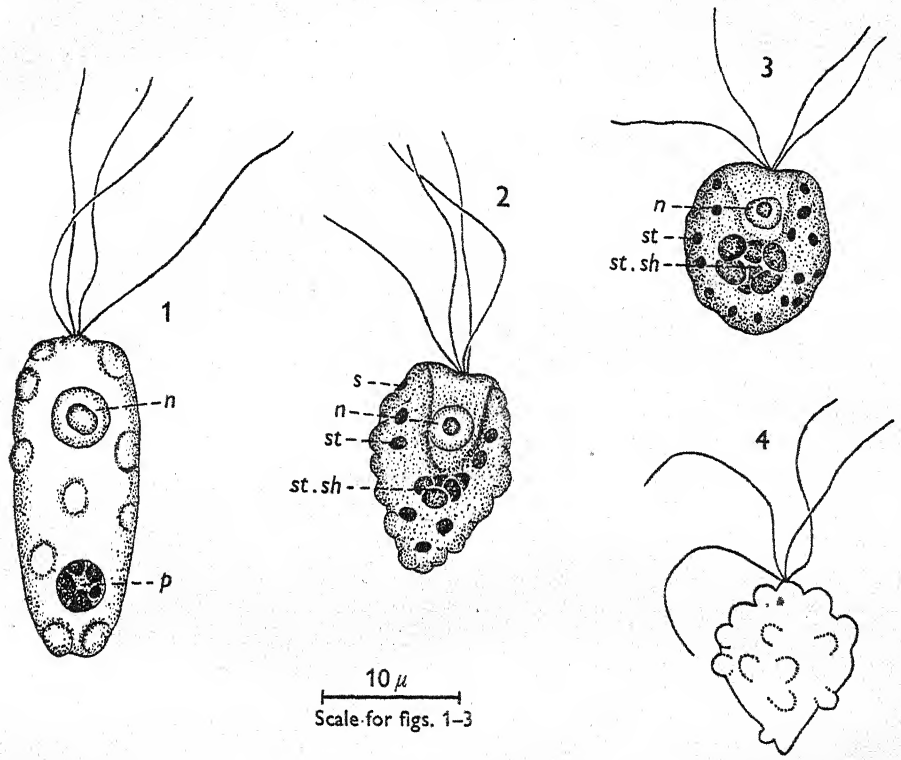
(*a*) *Pyramimonas botryodes* n.sp. This alga (Figs. 1–3) was collected in water in a horse's hoof-print on heavy clay, near Shenley, Hertfordshire, in May 1937. It was associated with several volvocalean forms, and the algae were present in sufficient quantity to colour the water green: the *Pyramimonas* was tolerably abundant, but it proved to be too delicate to keep in culture, disappearing within two or three days. No dividing cells were found, nor was any trace of reproduction observed. The plant has not been found subsequently.

The cells vary somewhat in shape, some being elongate and ovoid (Fig. 1), others pyriform (Fig. 2), and yet others almost as broad as long (Fig. 3). The surface of the naked protoplast is not smooth, as in other species of *Pyramimonas*, but somewhat verrucose, this constituting one of the most distinctive features of the species, and giving the cells a foamy or blistered appearance. The flagella are as long as, or somewhat longer than, the cell.

The chromatophore is cup-shaped, and without any anterior incisions such as are found in the chromatophores of most members of this genus. There is a conspicuous, anteriorly placed stigma; a single pyrenoid, with a prominent starch sheath composed of relatively large starch grains, lies posteriorly. A similar prominent starch sheath is found in *Pyramimonas delicatulus* Griff., the only other species of the genus with which I am familiar, and, to judge by illustrations, in other species as well. Isolated starch grains also occur in the chromatophore, and these are of relatively large size, although smaller, and sometimes much smaller, than those which compose the starch sheath (Fig. 3). The nucleus occupies the usual position in the cup formed by the chromatophore; it possesses a single, conspicuous nucleolus. No contractile vacuoles were observed.

This alga bears a resemblance, albeit a rather superficial one, to the motile zygote of *Chlamydomonas botryodes* which has been described by Strehlow (1929). This zygote remains motile for some days. It must be pointed out that species of *Chlamydomonas* were present in this Shenley collection, but I do not know if they included *C. botryodes*; in fact, I am doubtful if Strehlow's description of this species is sufficient to enable the plant

to be identified again with certainty. However, the zygote which this author describes and figures (cf. Fig. 4) has prominences which extend all over the surface of the cell, whereas in the *Pyramimonas* under present consideration they are usually absent from the anterior end. Further, in the *Pyramimonas*, there is a prominent, if small, stigma, whereas in the zygote of *Chlamydomonas botryodes* Strehlow emphasizes that the stigma is faint; nor does he mention a pyrenoid and associated starch sheath, features to which he is unlikely to have failed to draw attention, had they been as conspicuous as in *Pyramimonas botryodes*. Moreover, I am not aware of any species of *Chlamydomonas* which shows so conspicuous a starch sheath around the pyrenoid as is found in species of *Pyramimonas*.




Figs. 1–4.

Figs. 1–3. *Pyramimonas botryodes* n.sp. Fig. 1 is drawn more or less in surface view, to show wart-like protuberances; Figs. 2 and 3 are in optical section; Fig. 4, motile zygote of *Chlamydomonas botryodes* (after Strehlow). *n.* nucleus; *p.* pyrenoid; *s.* stigma; *st.* starch; *st.sh.* starch sheath.

There would thus appear to be good grounds for concluding that the alga which I have named *P. botryodes* is not the motile zygote of *Chlamydomonas botryodes*, but an undescribed species of *Pyramimonas*.

In possessing a cup-shaped chromatophore which is not cleft anteriorly, *P. botryodes* seems to be most closely related to *P. inconstans* Hodgetts; it differs from this species primarily in its verrucose surface; it is also slightly larger than *P. inconstans*.

*Pyramimonas botryodes* sp.nov. Forma cellarum a piro ad ovum variat, interdum latitudo longitudinem prope aequat, interdum latitudo plus dimidio minor quam longitudo: in superficie cuiusque cellae eminent nonnulla tubera rotunda: flagella aut aeque

longa ac cellae aut paullo longiora: chromatophorum formam calicis praebet sine fissuris anterioribus: ab anteriore parte, cellae stigma conspicuum habent, et a posteriore pyrenoidem magnum habent quod cingit quasi vagina quae e granulis amylaceis paucis et magnis consistit: cellae 12–23$\mu$ longae, 9–11$\mu$ latae.

Cell of variable shape, pyriform to ovoid, sometimes as broad as long, sometimes with a length exceeding twice the diameter: surface of cell bearing a number of rounded prominences: flagella as long as, or rather longer than, the cell: chromatophore cup-shaped without anterior clefts: cell with a conspicuous anterior stigma and a large, more or less posterior pyrenoid surrounded by a sheath of a few large starch grains: cells 12–23$\mu$ long, 9–11$\mu$ broad.

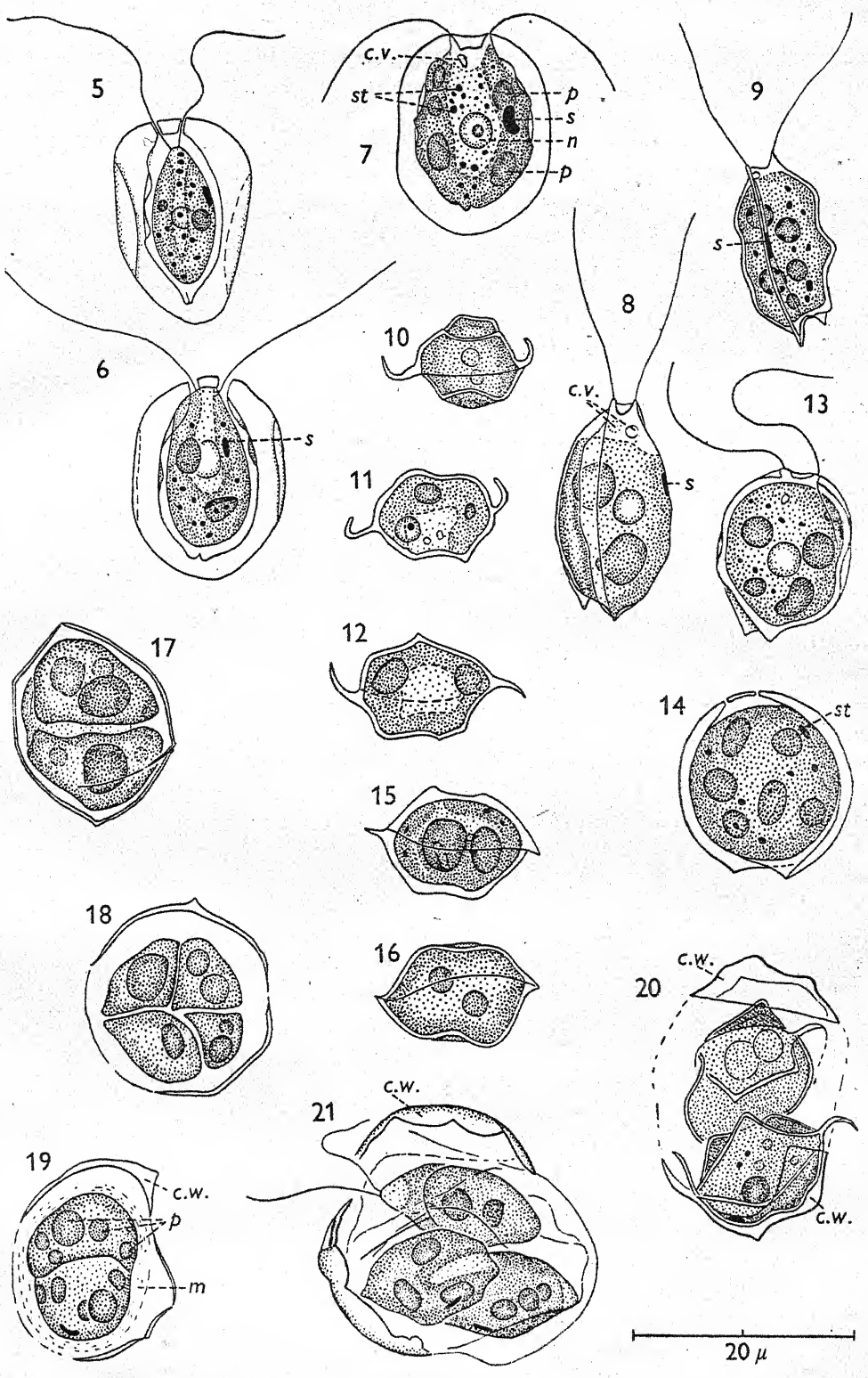
(*b*) *Pteromonas varians* n.sp. (Figs. 5–21). The alga on which this description is based was found in a roadside pond at Shenley, Hertfordshire, during late August and September 1938. It was not abundant, and was associated chiefly with species of *Chlamydomonas* and with *Phacotus lenticularis*. It was obtained from one small area of the pond, and was, so far as the pond margin is concerned, limited to this one place. Following heavy rainfall, with a consequent rise of water level, in late September and early October, it disappeared, or at least could not be found in samples which were collected at that time. The plant was not difficult to keep alive for some time: some specimens even remained alive for over a week in a centrifuge tube containing only 1–2 c.c. of water, and placed in a very reduced light. In spite of protracted observations, extending over some weeks, no trace of sexual reproduction was observed, although asexual reproduction took place during most of that period.

There are two well-marked types of vegetative cell; one (Figs. 5–12) has the wing-like expansion so characteristic of the genus, the other (Figs. 13, 14, 16) is without the wings. It is doubtful if the wingless form would be recognized as a species of *Pteromonas* if encountered alone, but in my material it was possible to trace gradations between the two forms, and to satisfy myself that the wingless forms are nothing more than older vegetative cells, nearing the time for asexual reproduction. The winged type, therefore, is to be regarded as the typical vegetative cell, and it is on this that the following description is based.

The cell, exclusive of the wing, is flattened, and about twice as broad along one transverse axis as along that at right angles to it: the length exceeds the greatest breadth. The conspicuous cell wall is produced into a wing, which lies in the plane of the longer transverse axis. The wing extends round both anterior and posterior ends of the cell, and is wide and thin; its shape is rather variable; the sides, anteriorly, may be rounded (Fig. 5), but are often truncated (Fig. 6); posteriorly, the wing is rounded; along the sides of the cell the wing may have a gently curved edge, corresponding to the curve of the cell, but frequently its lateral margins are straight (Fig. 5), when the wing appears

**Legends to Figs. 5–21**

Figs. 5–21. *Pteromonas varians* n.sp. Figs. 5–12, motile cells with prominent wings; 5–7 from the broad side, 8 and 9 almost from the narrow side, 10 from the posterior end, 11 and 12 in optical transverse section; the cell shown in Fig. 7 is probably near to the time for asexual reproduction, as the wing is much narrower than those of the cells shown in Figs. 5 and 6. Figs. 13–16, cells approaching the time of asexual reproduction; the lateral wings have almost or quite disappeared; 13 and 14 are from the broad side, 15 and 16 from the posterior end. Figs. 17–21, asexual reproduction; in 17 and 18 the contents have divided into four; in 19 the wall of the mother cell has split, and the mucilage surrounding the daughter cells is seen; in 20 and 21 the mother cell has split to liberate the zoospores. *c.v.* contractile vacuole; *c.w.* cell wall of mother cell; *m.* mucilage; *n.* nucleus; *p.* pyrenoid; *s.* stigma; *st.* starch.


5
c.v.
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Figs. 5–21.

to be narrower than usual; this straight-sided wing is caused by the bending of the two lateral margins, often in opposite directions, out of the plane of the wing (Figs. 5, 6), a feature readily discernible when the cell is viewed end on (Fig. 10), but easily overlooked when the cells are seen from the broad side, by reason of the transparency of the wing. The surface of the cell is not smooth, but is produced into a number of projections, which are sometimes gently rounded, sometimes angular (Figs. 7, 9, 12). Two of these projections lie posteriorly, in different planes, and when the cell is seen in optical section it appears to be prolonged posteriorly into a short tail, which is sometimes of sufficient length to reach nearly to the posterior margin of the wing. The two flagella, which are as long as, or rather longer than, the cell, are fairly widely separated as they pass through two prominent pores in the wing (Figs. 5, 6).

The cell contents are dense, and consequently rather difficult to elucidate. The chromatophore is of the usual cup-shaped type, generally comparatively massive, and somewhat variable in the relative proportions of its parts: it may not form a complete cylinder anteriorly, for it is sometimes cleft down one side (Figs. 11, 15), a condition which does not, however, appear to be common. Within the chromatophore lie from one to six or seven pyrenoids, with which are associated a small amount of starch, although a well-marked starch sheath has not been demonstrated. These pyrenoids vary, both in size and shape (Figs. 7, 19), and they are largely responsible for obscuring other details of the cells. There are usually numerous, small, ovoid starch grains scattered throughout the chromatophore. A flattened, elongated stigma lies slightly anterior to the middle of the cell, and along one of the narrower sides. Seen from the broad side, the stigma is frequently inconspicuous, as its edge is presented to the observer, but viewed at favourable angles it is prominent enough. The nucleus occupies the usual position in the hollow of the cup-shaped chromatophore, but in the living cell, or even in temporary preparations, it is exceedingly difficult to make out: it was demonstrated with iron-aceto-carmine, but without this reagent it was detected with certainty only in one cell. A nucleolus is often absent, or at least cannot be demonstrated, and this possibly explains the obscurity of the nucleus.

According to the Floras (e.g. Pascher, 1927; Smith, 1933), there are two contractile vacuoles in the cells of *Pteromonas*, but this is rarely so in *P. varians*. Observation of the vacuoles, no less than of other parts, is difficult because the cells are so dense, but in favourable instances it is possible to observe a vacuole, which contracts rhythmically, at the anterior end of the cell, just at the top of the hollow in the chromatophore. This is the only vacuole visible in most cells, although very rarely cells are seen which have two contractile vacuoles (Fig. 8).

When the time for asexual reproduction approaches, the wing decreases in size and gradually disappears (Figs. 13, 14, 16). This is held to be due to the increasing size of the protoplast, which is accommodated by gradual extension into the wings. If this explanation be the true one, it will be evident that the wing divides in the plane of the longer transverse axis, so as to make room for the enlarging protoplast; it might, therefore, be expected that such cells would be of considerably greater size than the winged vegetative cells. Some increase in size does occur, but not sufficient, it would seem, to account for the inclusion of the wings into the wall in the manner postulated; when split, however, the wings would be extremely thin, and a certain amount of contraction might well take place. In these more or less wingless cells the protuberances on the wall are less pro-

minent than in the vegetative cells, and are more gently rounded, although the posterior ones are still quite large. The cell contents become even denser, and the difficulties of studying the internal organization are correspondingly increased. The pyrenoids in such cells are numerous (six to seven), and usually large, and occupy much of the cell. Cells of this type are much less frequently seen than the winged cells, but stages may be traced showing the decreasing width of the wing, and thus connecting the two types of cell; such transitional types of cell are uncommon.

The denseness of the contents of the wingless cells suggests the accumulation of food reserves, and this is readily confirmed by the application of iodine, which produces a bluish, or more often, a dark, dirty brown coloration throughout the cell, the contents being almost completely obscured. While the winged cells may contain no more than one or two pyrenoids (Fig. 5), in wingless cells these bodies are nearly always more numerous (Fig. 13), numbering six or seven, and it is concluded that the pyrenoids may increase in number with the approach of asexual reproduction.

Cells which showed division of the protoplast to form daughter cells were rarely seen; in such as were found, the protoplast had divided into four, by two divisions at right angles to one another (Fig. 18). Later stages, showing mother cells containing two or more, often four, daughter cells, were encountered, although it was not possible to ascertain if the two-celled types were derived from a protoplast which had divided once, or if there are always two divisions, the two-celled type arising by abortion of half the daughter cells. The daughter cells are walled and possess a prominent wing, of which the two edges are folded more or less at right angles to the inner part (Fig. 20). Before the daughter cells are completely formed, the two valves of the mother cell have separated, and the new cells lie in a mass of colourless material, which is readily stained by very dilute methylene blue, and which is, doubtless, mucilage. The separation of the two valves is by no means as neat and precise as in *Phacotus*, but it serves the same end, and the daughter cells gradually move away.

In the culture in which this *Pteromonas* was most abundant, cells containing two or four daughter cells were found almost entirely on parts of the glass which had become covered with a green film which consisted largely of palmelloid *Chlamydomonas*. In many instances the daughter cells of the *Pteromonas* were immobile, and sometimes, in such cells, no trace of flagella could be observed. It seems not improbable that at this stage the plant sometimes passes into a resting condition, from which the daughter cells do not emerge until conditions are favourable for a motile existence. If this be so, the stage may be regarded as an incipient palmelloid condition, in which the four daughter cells remain in a mass of mucilage under unfavourable conditions, although, as far as my observations go, the cells do not divide further. A similar explanation may hold for the daughter cells of *Phacotus lenticularis*, which were likewise observed, in the same culture, to be, not uncommonly, inactive.

It is with some hesitation that the alga under consideration is described as a new species. To the present author the systematics of the genus *Pteromonas* appear at best confused and unsatisfactory, and there would seem to be several 'forms' with specific rank, which cannot at present be regarded as good species. Nevertheless, this Shenley form does appear to be sufficiently distinct to deserve specific distinction. In the shape of its wing it somewhat resembles Playfair's *P. angulosa* var. *vexiliformis*, especially if it be recognized that the flattened lateral margins of the wing in this variety may be due merely to an

upturning or a downturning of the edges, so that they lie in a plane at right angles to the main part of the wing: beyond this point, however, as far as may be judged from Playfair's (1918) rather inadequate description, the two are distinct, and it may be noted that in Playfair's variety the wing does not extend over the anterior end of the cell. Protuberances are present on the cells of both *P. Golenkineana* and *P. aculeata*, but, to judge from the illustrations of these two species, the protuberances are much more pointed than in the present species, which further differs from *P. aculeata* in the shape of its wings. There seems little doubt that the present species is most closely related to *P. Golenkineana*, but in the latter the wing is circular in outline, the cell of a more rectangular shape and without 'tails' (cf. Golenkin, 1892, Figs. 1, 3), and the protuberances on the surface of the cell more pointed. Further, *P. varians* is not more than two-thirds of the size of *P. Golenkineana*.

*Pteromonas varians* sp.nov. Cellae platyformes sunt et alterum axem transversum prope bis longiorem altero habent; longitudo maximam latitudinem superat: murus cellae conspicuus et in alam productus in eadem planitie ac longior axis transversus; haec ala quae totam cellam cingit, lata quidem est sed tenuis; in anteriore parte ala aut rotunda aut truncata, in posteriore parte rotunda; margines laterales alae interdum curvatae sed saepe rectae, id quod evenit proptereaquod margines e planitie alae nonnunquam curvantur: superficies cellae habet tubera lĕviter curvata, quorum duo sunt in posteriore parte: flagella sunt aeque longa ac cellae vel etiam longiora: chromatophorum formam calicis habet et in uno latere anteriore nonnunquam fissuram praebet: pyrenoides 1–7 saepe magnae: stigma quod formam disci praebet non in media superficie cellae iacet sed paullum versus anteriorem partem, et e regione unius ex angustis lateribus: in anteriore parte est unum vacuolum quod se contrahit, rare duo vacuola: aut 2 aut 4 zoospora fiunt in cella parente; haec nullam habet alam; cellae quae fiunt solent alam conspicuam praebere; zoospora liberantur cum murus cellae parentis in duas partes finditur; haec fissura quae in eadem planitie fit ac alae, satis directa est: longitudo cellae cum ala 18–19$\mu$; sine ala 14–15$\mu$; latitudo cellae cum ala 14–18$\mu$; sine ala 7–12$\mu$; altitudo 8$\mu$.

Vegetative cell flattened, with one transverse axis about twice as long as the other; length exceeding greatest breadth: cell wall prominent, and produced into a wing in the plane of the longer transverse axis; wing extending all round cell, wide and thin; anteriorly rounded or truncated, posteriorly rounded; lateral margins of wing sometimes curved, but often straight, owing to bending of margins from plane of wing: surface of cell beset with gently rounded protuberances, of which two are posterior: flagella as long as, or rather longer than, cell: chromatophore cup-shaped, sometimes cleft down one side anteriorly: pyrenoids from one to seven, often large: stigma oval, flattened, lying slightly anterior to middle of cell and along one of the narrow sides: a single anterior contractile vacuole, rarely two: two or four zoospores produced in the mother cell, which is not winged; daughter cells with prominent wing; zoospores liberated by splitting of wall of mother cell into two, fairly regularly, along plane of wings: length, including wing, 18–19$\mu$, excluding wing, 14–15$\mu$; breadth, including wing, 14–18$\mu$, excluding wing 7–12$\mu$; breadth in plane at right angles to wing 8$\mu$.

## II. The genera *Furcilla* Stokes and *Pseudofurcilla* n.g.

The genus *Furcilla** was erected by Stokes (1890) for the reception of a colourless flagellate, *F. lobosa*, which was found in an infusion of decaying vegetable matter. Stokes appears to have had some difficulty in placing the genus systematically; he considered that it showed affinities with *Amphimonas* Dujardin, and especially with *Goniomonas* Stein. Stokes did not state if the organism possessed a cell wall, although it is to be inferred from his paper that it did not, especially as he regarded its affinities as with the two genera just named, both of which are naked. Following Stokes, Lemmermann (1910) placed the genus in the Protomonadineae along with *Amphimonas* and *Goniomonas*, but most authors now refer it to the Volvocales (Pascher, 1927; Printz, 1927; Smith, 1933; Fritsch, 1935). Both Printz and Pascher place the genus among the Polyblepharidaceae, i.e. as one of the naked volvocalean genera, and the latter authority adds another species, *Furcilla trifurca*, presumably a naked, colourless form. Since the appearance of the works of Pascher and Printz, Skuja (1927) has described in some detail an organism which he has referred to *F. lobosa*, although it differs from Stokes's species in several respects. Fritsch (1935) has suggested that the genus is, perhaps, a member of the Chlamydomonadaceae, and Smith (1933) places the genus in this family. Since Skuja states that the cells are walled, there seems to be no doubt that the genus, if volvocalean, is correctly placed in the Chlamydomonadaceae and not in the Polyblepharidaceae.

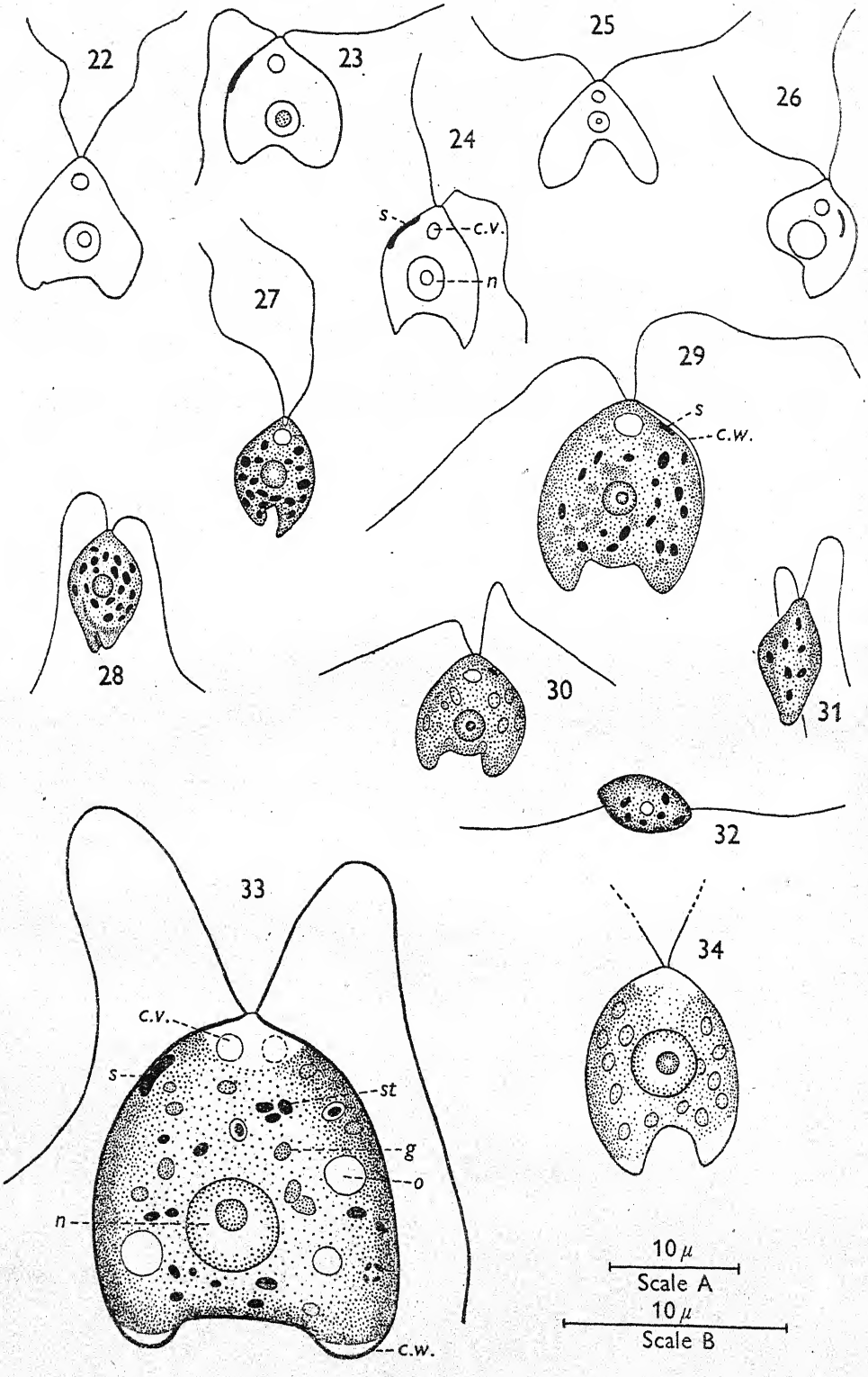
Early in 1937 I received from Darlington, from Miss R. E. Dowling, some greenish water in which twigs of *Aesculus* had been kept; the green colour was caused chiefly by a flagellate alga, so closely resembling that figured by Skuja (1927) as to leave no doubt that it was a closely related form. The resemblance to Stokes's species, or to *Furcilla trifurca* is not marked, and I am of the opinion that the plant described by Skuja, and the one which I have studied, must be regarded as distinct from *Furcilla* and worthy of generic rank. It is, therefore, proposed to name these plants *Pseudofurcilla*, and Skuja's plant thus becomes *P. lobosa*.

The specimens of *Pseudofurcilla* which I studied were roughly hastate (Figs. 22–34), flattened along one transverse axis and tapering to a short papilla anteriorly. There is considerable variation in the shape of the cells; normally the posterior processes are rather short and rounded (Figs. 22, 33), but in some cells they are somewhat pointed (Figs. 24, 34); less frequently they are half as long as the cell (Fig. 25), and sometimes, owing to the greater development of one of the lobes, the cells are asymmetrical (Fig. 26). Along one transverse axis the cells are at least half as broad as long, and sometimes the breadth along this axis is equal to the length of the cell: along the transverse axis at right angles to this the cell is flattened (Figs. 31, 32); along this narrower axis the cell may be somewhat swollen in the middle (Fig. 31), but such a median swelling is not invariable, nor have I ever observed a cell, as seen from the anterior end, to have the regular four-lobed appearance of the *Furcilla* cell figured by Stokes.

The two flagella arise on opposite sides of the papilla, near its tip, and are as long as, or rather longer than, the cell.

The protoplast is surrounded by a distinct cell wall which gives a positive reaction for cellulose when treated with iodine and sulphuric acid. This wall is not easily discerned

* Both Skuja (1927) and Smith (1933) refer to this genus as *Furcilia*.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10 μ
Scale B

Figs. 22–34.

in living cells, but is often rendered conspicuous in cells which have been killed, owing to the slight plasmolysis which is sometimes produced by the toxic agent.

The cells have a pale green colour, paler than is usual in a green volvocalean flagellate. Because of the faint pigmentation, it was not possible to ascertain if there was a discrete chromatophore; in most cells the chlorophyll appeared to extend throughout the cell, but in one or two specimens there was some indication that the chromatophore was somewhat cup-shaped (Fig. 34). The anterior end of the cell appeared normally to be free from chlorophyll (Fig. 33). Each cell possesses a prominent, elongated stigma, which is situated anteriorly along one of the narrower sides, and which abuts on to a thin area of the wall. Except for this region, and on the papilla, where it is extremely thin and difficult to make out with certainty, the wall is evenly thickened all over the cell. Anteriorly there are two relatively large contractile vacuoles, which in living cells may easily be seen contracting and expanding alternately. I am inclined to think that the presence of these vacuoles may obscure the chlorophyll at the anterior end of the cell, and that the pigment may actually be diffused throughout the whole cell. The nucleus is large, and has a prominent nucleolus; it normally occupies a position somewhat posterior to the middle of the cell. There are no pyrenoids, but numerous scattered starch grains are present, and sometimes larger globules of an undetermined nature, some of which are probably oil globules.

The cells have a length of 8·2–10·7 (15)$\mu$, a breadth of from 6·4 to 10·0 (14)$\mu$, and a thickness of 4·0–5·0$\mu$. The cell, which had a length of 15$\mu$ and a breadth of 14$\mu$, was an exceptionally large specimen in the material which I examined; few of the cells exceeded 8·5$\mu$ in length and 8$\mu$ in breadth.

The cells move with a peculiar vibritil emotion. Progression is always anterior end foremost.

I was not fortunate enough to discover any reproductive stages in my material. Skuja (1927), however, observed asexual reproduction, the contents of the mother cell dividing into four zoospores, as well as sexual reproduction by motile, anisogamous gametes.

The plants which Skuja found near Riga were colourless, but in other respects appear to resemble those which I have just described. Later, Skuja obtained specimens from the Ålandinseln which had a small lateral stigma; these latter plants he named provisionally *F. lobosa* f. *stigmatophora*; the majority had a faint green colour. Skuja's experimental cultures led him to conclude that the green plants develop in the light, while those in darkness are either colourless or but slightly green.

The question now arises as to whether the Darlington plants, in which all the cells were pigmented, are sufficiently different from the plants described by Skuja to be worthy of specific rank. In shape the plants are similar, the only difference being that the papillate anterior end is less pronounced in the Darlington plants than in Skuja's material. As to size, Skuja gives the dimensions as 10–15$\mu$ long, 8–14$\mu$ broad and 8$\mu$ thick; his plants were thus considerably larger than those which I studied, of which the dimensions

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**Legends to Figs. 22–34**

Figs. 22–34. *Pseudofurcilla lobosa* (Skuja) Jane, f. *britannica*. Figs. 22–26, outline drawings of cells to show variation in shape; Figs. 27–34, cells showing details of structure, 31 is viewed almost from the narrow side, 32 from the posterior end, the rest from the broad side, except 28, which is viewed somewhat obliquely. Figs. 29, 33, 34, scale B, remainder scale A. *c.v.* contractile vacuole; *c.w.* cell wall; *g.* granules of unknown composition; *n.* nucleus; *o.* oil globules? *s.* stigma; *st.* starch grains.

rarely exceeded 8·5$\mu$ long by 8$\mu$ broad. Nevertheless, one or two larger specimens were observed—that figured in Fig. 33 was 15$\mu$ long by 14$\mu$ broad, and thus as large as the biggest specimens observed by Skuja. There remains the stigma, large in the Darlington material, absent from Skuja's Riga plants, and small in his Ålandinseln specimens. While the presence or absence of an eyespot, and even the shape of this organ, has been used as a specific distinction, I feel some hesitation in adopting such a course in the present instance, since our knowledge of these flagellates is scanty. I therefore place all three plants in Skuja's species, which now becomes *Pseudofurcilla lobosa* (Skuja) Jane, with three distinct forms, viz. the colourless form without a stigma, which I propose to designate forma *hyalina*, the colourless or pale green form with a small stigma, which Skuja has already referred to as forma *stigmatophora*, and the form with a large, rod-shaped stigma and possibly always pigmented, which I name forma *britannica*.

*Pseudofurcilla* gen.nov. Cellae sunt platyformes et satis hastatae, cum duobus flagellis inter se paribus: cum muro celluloso: chromatophorum quod nonnunquam abest, minime definitum: interdum novae cellae asexualiter fiunt, nam in 4 biflagellata zoospora cella quaeque dividi potest: interdum sexualiter fiunt per biflagellatas gametas quarum altera altera paullo minor; zygotes quadriflagellata quae tempus aliquod movetur, conquiescit tandem et lēvem murum facit.

Cells flattened, more or less hastate, with two equal flagella: enclosed in a cellulose wall: chromatophore, if present, ill defined: asexual reproduction by division of cell into four biflagellate zoospores: sexual reproduction by biflagellate gametes, of which one is slightly smaller than the other; quadriflagellate zygote motile for some time, later coming to rest and secreting a smooth wall.

Only species *P. lobosa* (Skuja) Jane; characters as genus. This species has three forms distinguished as follows:

A. Without stigma — f. *hyalina*.
B. With stigma.
  *a*. Stigma small — f. *stigmatophora*.
  *b*. Stigma large, rod-like — f. *britannica*.

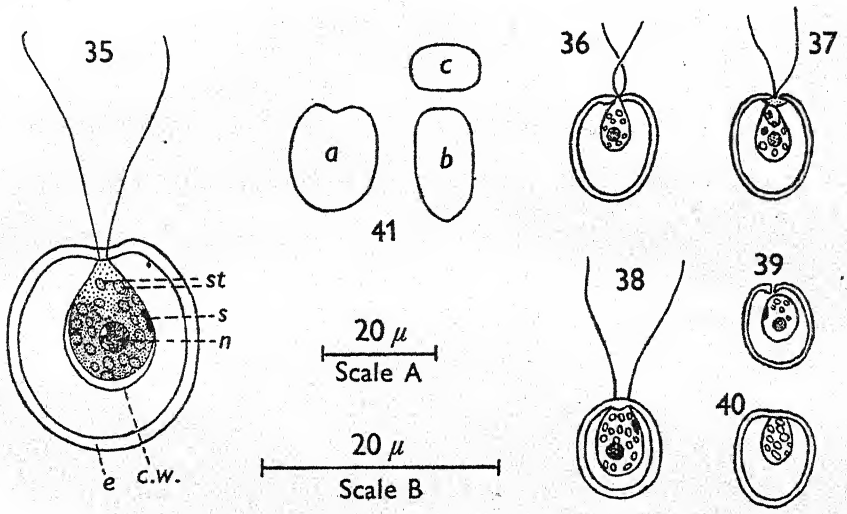
It has been seen that certain details are lacking in Stokes's description of *Furcilla*, but the following tentative description of the genus is probably accurate:

*Furcilla* Stokes. Cells hastate, with two equal, or nearly equal, flagella: naked: colourless: reproduction unknown, probably by longitudinal division of the cell into two daughter cells.

Until such time as more is known of the genus, I am of the opinion that *Furcilla* is best referred to the Protomastigineae. I have no doubt that *Pseudofurcilla* may be correctly referred to the Chlamydomonadaceae; its affinities seem to lie with genera like *Brachiomonas* and *Lobomonas*. It is possibly even more closely related to, if not identical with, the little known genus *Chlorotriangulum*, which has been described by Kufferath (1914). While the separation of *Pseudofurcilla* from *Furcilla* may meet with general acceptance, I can scarcely hope to escape criticism for not referring *Pseudofurcilla* to *Chlorotriangulum*. I do not, however, consider Kufferath's description of *Chlorotriangulum* adequate, and I do not think that any useful purpose is served in referring new plants to a genus which cannot be identified with certainty. Should future work firmly establish the limits of the genus *Chlorotriangulum*, it may well be that *Pseudofurcilla* will become a synonym.

### III. *Coccomonas platyformis* n.sp., and *C. orbicularis* Stein

The alga for which I propose the name *Coccomonas platyformis* was taken from a dune slack at Newborough, Anglesey, early in May 1942. The most conspicuous feature of the cells was that one transverse axis was little more than half the length of that at right angles to it, so that the cells were markedly flattened, in this respect resembling the allied genus *Pedinopera* rather than *Coccomonas* (Figs. 35–41). There was some variation in the shape of the cells, due to the varying ratio between the longer transverse axis and the longitudinal axis: viewed from the broad side, in optical section the anterior end is emarginate (Figs. 35, 41). The flagella are 1–1½ times as long as the cell. The protoplast is similar to that of *C. orbicularis*, with a cup-shaped chromatophore, a large posterior pyrenoid and an elongate stigma which, while in the anterior half of the protoplast, may




Figs. 35–41. *Coccomonas platyformis* n.sp. Figs. 35–40, cells viewed from broad side; Fig. 41, outline drawing of envelope, *a* from broad side, *b* from narrow side, *c* from anterior end. Fig. 35 scale B, remainder scale A except 41, which is from a freehand sketch. *cw.* cell wall; *e.* envelope; *n.* nucleus; *s.* stigma; *st.* starch grains.

be situated almost in the middle of the cell (Fig. 35). Numerous small, ovoid starch grains are scattered throughout the chromatophore, but there is no conspicuous starch sheath around the pyrenoid. No contractile vacuoles were observed in the cells, but as the envelope, as is usual in older cells of species of this genus, was stained with iron salts, I do not consider that failure to observe contractile vacuoles should be regarded as conclusive evidence for their absence. No young cells were observed in my collection, for I did not see plants with the *bâtonnets* of calcareous material, which appear to characterize the young cells of *Coccomonas* (cf. Conrad, 1930).

Reference to the literature on the genus *Coccomonas* leaves the impression that our knowledge of these plants is confused. Stein (1878), who erected the genus for the reception of *C. orbicularis*, figured several plants of different shapes, but none with an emarginate anterior end. Pascher (1927) figures three individuals as *C.* ? *orbicularis*, which differ from those in Stein's illustration principally in their broad, emarginate

anterior end; Pascher refers to the variability of this species. Conrad (1930) attempted to monograph the genus, admitting five species, of which he appears to have seen four. One character which he uses as diagnostic for *C. orbicularis* is the shape of the envelope, which, he claims, is elliptical in transverse section in *C. orbicularis* and circular in the other species. Stein (1878), however, in his description of *C. orbicularis* (p. 54), writes: 'Ganz ebenso wie *Phacotus* verhält sich meine Gattung *Coccomonas*...nur ist hier die Hülse drehrund...wie bei *Trachelomonas*.' There can be no doubt, therefore, that *Coccomonas globulifera* Stein is circular in transverse section, and that Conrad's limitation of the species to plants which are elliptical in transverse section is inadmissible. Whether it is justifiable to enlarge the limits of this species to include forms which are elliptical in transverse section is another matter, and one on which there is likely to be a diversity of opinion, especially as it seems evident that *C. orbicularis* is a variable plant. In my Newborough material the flattening was a conspicuous and a constant feature. Moreover, these plants were smaller than *C. orbicularis*, having a total length of 16–20$\mu$ and a width of 13–16$\mu$, as compared with a length of 23–27$\mu$ and a width of 17–21$\mu$ in *C. orbicularis*.* Nevertheless, it would not be wise to attach much importance to these figures, for Conrad gives the size of his plants, which are, perhaps, the same species as my Newborough plants, as from 20 to 50$\mu$ long.

In my opinion the flattened cells are sufficiently distinct from those of *C. orbicularis* Stein to be worthy of specific distinction, and I propose to designate these Newborough plants *C. platyformis*.

*Coccomonas platyformis* sp.nov. Cellae platyformes quarum alter transversus axis circa dimidio minor altero: e latiore latere spectanti, cella formam praebet rotundam aut ellipticam, excepto quod in margine anterioris partis est intermissio quaedam vel quasi cavea: cellae longae 16–20$\mu$; latae 13–16$\mu$; altae circa 8$\mu$.

Cells flattened, with one transverse axis about twice as long as the other: except for emarginate anterior end, cell, viewed from the broader side, circular or elliptical: 16–20$\mu$ long, 13–16$\mu$ broad, *c.* 8$\mu$ thick.

I desire to thank Mr C. S. Chapman for rendering my English descriptions into Latin.

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* These measurements are calculated from Stein's figures. No measurements of *C. orbicularis* appear to have been recorded.

# STUDIES IN ATMOSPHERIC POLLEN

## I. A DAILY CENSUS OF POLLENS AT CARDIFF, 1942

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(With 3 figures in the text)

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### Introduction

It has for some years been apparent, at least to students of peat stratigraphy and to medical allergists, that a detailed study of the pollen precipitation in Britain was desirable: to students of peat, because the pollen deposit of to-day is the key to that of yesterday; to medical allergists, because only in the light of knowledge of what pollens are in the air and in what quantity will they be able to forecast which kinds will be likely to cause allergic reactions. In spite, however, of its geological and medical implications the composition of the pollen content of the atmosphere is essentially a botanical problem and it has here been treated purely as such.

Charles Blackley of Manchester, who first proved that pollen is the cause of hay fever, was also the first to employ what is now called the gravity method of comparing the numbers of pollen grains in the air at different times. He trapped pollen grains on a horizontal adhesive surface and counted the numbers so obtained per unit area in unit time. Blackley's method was revived about a quarter of a century ago in the U.S.A. by W. Scheppegrell and has been employed very widely also by other workers in that country, notably by O. C. Durham in his researches on the incidence of ragweed (*Ambrosia*) pollen, and more recently elsewhere. The subject has recently been reviewed by Vaughan (1939, p. 465). Among the more recent papers on atmospheric pollen that by Phillips (1941) on Australian pollens may be mentioned. This worker relied mainly on slides exposed vertically. Very few observations on atmospheric pollen have been made in Europe since Blackley's. Bertsch (1935) published the results of exposing adhesive slides at Ravensburg: he confined himself to tree pollens and to the spring and early summer

months. Lüdi & Vareschi (1936) working at Davos, Switzerland, in 1934–5, caught pollen in open dishes containing glycerin: as a rule they changed the receptacle not more often than once a week; they distinguished twenty-three types of pollen, but their curves show the variations month by month in the deposition only of various genera of tree pollens, and grass and sedge pollens respectively (see also Lüdi, 1937). Erdtman (1938) has published direct determinations of the pollen content of the atmosphere at Västerås, Sweden, using his vacuum-cleaner method, but has not published systematic daily observations on the point.

## General procedure

The survey here described took the form of continuous day-to-day observation of the pollen precipitation at Llandough Hospital, Cardiff, during the period 30 November 1941 to 31 December 1942. A 3 × 1 in. microscope slide, which had been previously prepared by smearing the surface with a very thin covering of melted glycerin jelly stained with basic fuchsin, was put out every morning at 10 a.m. in an apparatus designed for this purpose.

The apparatus in which the slides were exposed consisted of a light metal box 18 in. square by 10 in. deep with a vertical tail-piece attached to one corner, and at the bottom of the opposite corner an opening made by cutting away parts of two adjacent sides each measuring 5 × 4 in. Near the tail-piece were a series of small holes in the box which allowed exit for the air. The whole apparatus was set up like a weather vane, pivoted on a vertical rod so that it rotated freely and the opening always faced the wind.

The slide was placed horizontally just inside the opening so that it was protected from the rain but a free current of air flowed over it; it was fixed in position by means of a spring clamp at its proximal end.

The design of the apparatus was purely empirical and no claim is made that it yields results which bear any numerical relation to the actual pollen content of the atmosphere. No exhaustive tests were carried out to determine the relation between the actual catch per unit area inside the apparatus and that in the open: a few experiments showed that there was a very distinct loss due to roofing-in, but obviously under British conditions the slide must be protected. In our 1943 experiments we have used a simpler and (probably) better form of apparatus but not a theoretically ideal one, which still remains to be designed.

After 24 hr. exposure in the apparatus the slide was removed and examined with a hand lens and any pieces of grit or sand were removed with a mounted needle—thus allowing the coverslip to be mounted evenly. The slide was then warmed to remove any condensation moisture and was mounted with a $\frac{7}{8}$ in. coverslip using one drop of glycerine jelly. We observed a tendency for pollen grains to be carried toward the edge of the coverslip during mounting: if too much jelly were used some pollen might be carried from underneath the coverslip by the excess jelly. Care was taken therefore to use just sufficient jelly on the coverslip and no more. Because of the uneven distribution of the pollen after mounting, we have considered it advisable to count the whole area of the slide under the coverslip rather than any specified smaller area. All manipulation of the slide was carried out in such a way as to minimize the risk of chance contamination: in particular the slides were prepared before, and mounted after, exposure, in a laboratory into which no flowers were allowed to be brought.

## Site and surroundings of the pollen observation station

The whole apparatus was placed on a projection above the roof of the operating theatre, and therefore on the extreme north-east corner of the Hospital. The projection was $7\frac{1}{2}$ ft. above the general level of the roof and 22 ft. in all above the ground. The position thus selected was the most open that could be found in the neighbourhood, being completely exposed on all sides except for the wall of the main building, which is 18 ft. higher and stands 84 ft. away.

Llandough Hospital (Fig. 1) stands at an altitude of 200 ft. toward the eastern end of a narrow spur of elevated ground running W.N.W.–E.S.E. in the direction of the Bristol Channel, 2 miles away. Westwards the land rises gently and the ridge of which the Hospital spur forms a part rises ultimately at Cock Hill, Leckwith, to 375 ft. The pollen observation station therefore occupies an open elevated situation and is not overlooked by higher ground in the immediate vicinity.

The vegetational setting of the Hospital is indicated in the sketch map (Fig. 1). The Hospital site itself (apart from the buildings) is occupied mainly by grassy vegetation more or less modified by disturbance. About 35 % of the cultivated land within $1\frac{1}{2}$ miles radius of the Hospital is arable, the remainder under grass, the great bulk of which is mown for hay.* Haymaking began before the middle of June and the greater part of the grass in the district concerned had been cut by 1 July. The neighbouring woods are developed on a variety of geological formations all of which either consist lithologically mainly of limestone, or are highly calcareous marls. The dominant tree species throughout are ash, oak, maple and hazel. *Ulmus glabra* is common especially in Cwm George; both it and *U. procera* occur frequently as hedgerow trees. Other species, the local distribution of which is relevant, are referred to in a later section.

## Identification of pollens

We have derived a certain amount of assistance from Wodehouse's *Pollen Grains* (Wodehouse, 1935); we find, however, that his illustrations, while in some cases they serve to elucidate the details of pollen grain structure, seldom present a view of the grain as normally seen under the microscope and their usefulness for purposes of identification is limited. Many families likely to occur in British pollen grain are of course entirely omitted by this author. We have referred constantly to Zander's book (Zander, 1935): this contains a large fund of information on pollen which is unavailable elsewhere. Zander's more highly magnified photographs ($\times 450$) are exceedingly useful, the others ($\times 160$) much less so. Neither descriptions nor figures however can take the place of comparison with authenticated material: we have made use throughout of a collection of standard pollen slides prepared by the simple technique described by Wodehouse, using basic fuchsin as for our exposed slides. This method gives for the most part grains in the fully swollen condition: a small proportion however remain unswollen and therefore mostly unidentified.

Some pollens may be identified generically, others can be recognized only by familial characters: very few can be attributed to a particular species. We have therefore grouped

* We are indebted to Messrs J. H. Hamilton, B. T. Rees and K. L. Richards for information on the 1942 distribution of grassland and arable land in the district.

them in our tables under the headings of various 'types' not all of which are of the same taxonomic grade.

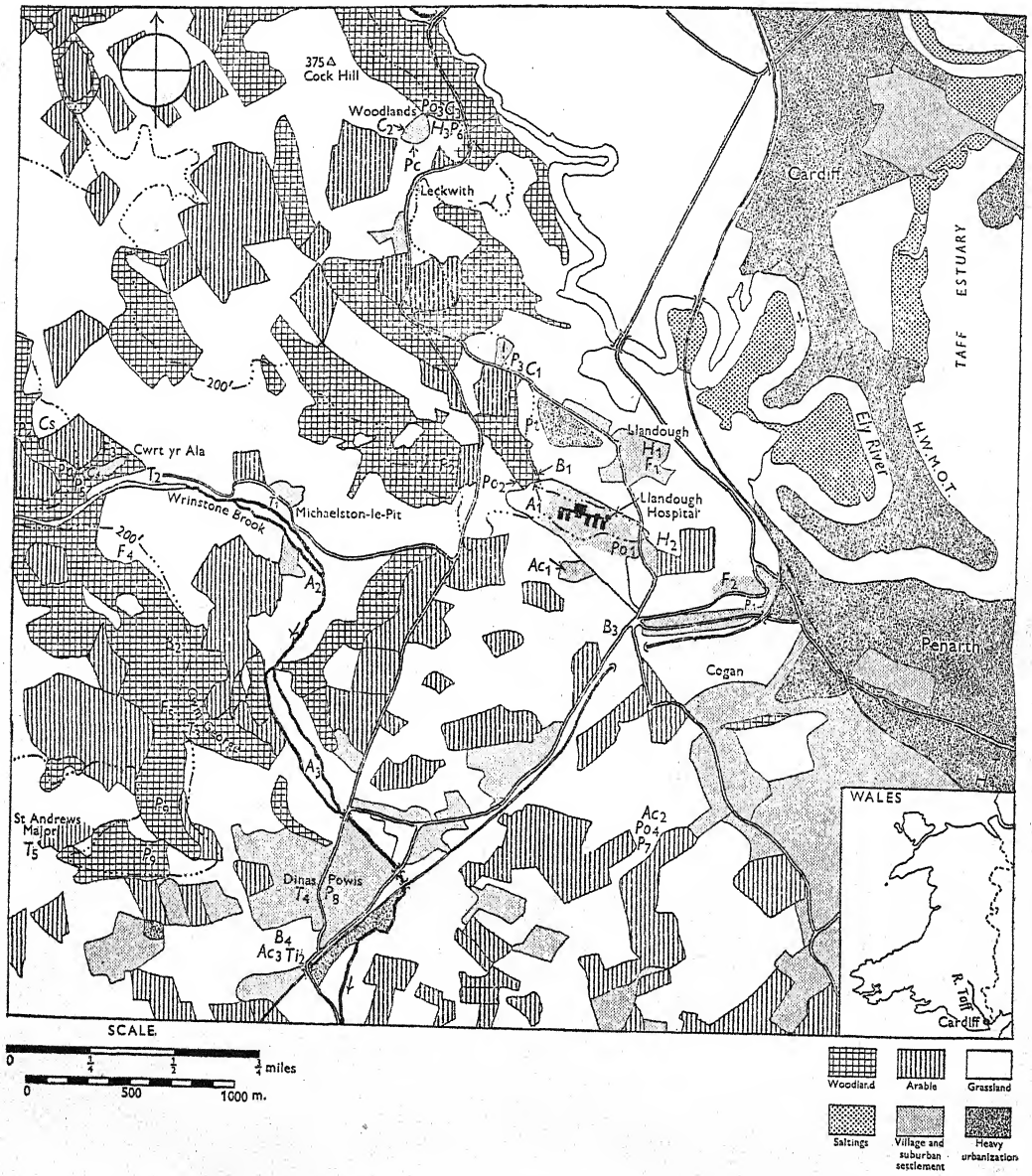



Fig. 1. Sketch map of the area surrounding Llandough Hospital, Cardiff. The sites of individual trees or groups of trees of the less common species are indicated thus; *$A_1$, $A_2$, $A_3$, Alnus*; *$Ac_1$, $Ac_2$, $Ac_3$, Acer Pseudoplatanus*; *$B_1$, $B_2$, $B_3$, $B_4$, Betula*; *$C_1$, $C_2$, $C_3$, $C_4$, Carpinus*; *Cs, Castanea sativa*; *$F_1$, $F_2$, $F_3$, $F_4$, $F_5$, Fagus*; *$H_1$, $H_2$, $H_3$, $H_4$, Aesculus Hippocastanum*; *$P_1$, $P_2$, $P_3$, $P_4$, $P_5$, Pinus silvestris*; *Pc, Picea Abies*; *$Po_1$, $Po_2$, $Po_3$, Populus serotina*; *Pa, Platanus acerifolia*; *$T_1$, $T_2$, $T_3$, $T_5$, Taxus baccata*; *Pt*, site for *Poterium Sanguisorba*. See also text.

## The detailed results

The results of the systematic analysis of the daily pollen catch throughout the year are summarized in Table 1. Graphs showing the day-to-day variation in rate of deposition of each of the principal anemophilous types are reproduced in Fig. 2 (tree pollens) and

Table 1. *Pollen deposition at Llandough Hospital, Cardiff*, 1942. *Total numbers of grains counted on* $\frac{7}{8}$ *in. square* (=5 *sq. cm.*)

| Type of pollen | Jan. | Feb. | Mar. | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Annual totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Clematis(e)** | — | — | — | — | — | — | 3 | 8 | — | — | — | — | 11 |
| *Thalictrum* | — | — | — | — | — | 1 | 14 | — | — | — | — | — | 15 |
| Other Ranunculaceae(*e*) | — | — | — | 2 | 11 | 32 | 17 | 4 | 3 | — | — | — | 69 |
| Papaveraceae(*e*) | — | — | — | — | — | 12 | — | — | — | — | — | — | 12 |
| Cruciferae(*e*) | — | — | — | — | 14 | 26 | 194 | 8 | — | — | — | — | 242 |
| Caryophyllaceae | — | — | — | — | 1 | — | — | — | — | — | — | — | 1 |
| *Tilia(e)* | — | — | — | — | — | — | 8 | 1 | — | — | — | — | 9 |
| *Impatiens(e)* | — | — | — | — | — | — | 10 | — | — | — | — | — | 10 |
| *Ilex(e)* | — | — | — | — | — | 2 | — | — | — | — | — | — | 2 |
| *Aesculus(e)* | — | — | — | — | 10 | — | — | — | — | — | — | — | 10 |
| *Acer(e)* | — | — | — | 5 | 18 | — | — | — | — | — | — | — | 23 |
| Leguminosae(*e*) | — | — | — | 1 | 2 | 6 | 8 | 5 | — | — | — | — | 22 |
| *Spiraea(e)* | — | — | — | — | — | — | 7 | 6 | 12 | 2 | — | — | 27 |
| *Poterium* | — | — | — | — | 2 | 1 | 1 | — | — | — | — | — | 4 |
| Other Rosaceae(*e*) | — | — | — | 3 | 23 | 9 | 17 | 3 | — | — | — | — | 55 |
| *Ribes(e)* | — | — | — | 1 | 7 | — | — | — | — | — | — | — | 8 |
| *Epilobium angustifolium(e)* | — | — | — | — | — | — | 1 | — | — | — | — | — | 1 |
| Umbelliferae(*e*) | — | — | — | — | 29 | 72 | 16 | 29 | 9 | 1 | — | — | 156 |
| *Hedera Helix(e)* | — | — | 1 | — | — | — | — | — | 3 | 12 | — | — | 16 |
| *Sambucus nigra(e)* | — | — | — | — | — | 74 | 12 | — | — | — | — | — | 86 |
| *Viburnum Tinus(e)* | 2 | — | 1 | 1 | — | — | — | — | — | — | — | — | 4 |
| Rubiaceae(*e*) | — | — | — | — | — | — | 3 | — | — | — | — | — | 3 |
| Cichorieae(*e*) | — | — | — | 1 | 5 | 7 | 3 | 5 | 3 | — | — | — | 24 |
| *Artemisia* | — | — | — | — | — | — | — | 66 | 19 | 4 | — | — | 89 |
| Other Compositae(*e*) | — | 3 | 2 | 2 | 6 | 13 | 9 | 5 | 9 | 37 | 1 | — | 87 |
| Ericaceae(*e*) | — | — | — | — | — | — | — | — | 1 | — | — | — | 1 |
| Primulaceae(*e*) | — | — | — | — | — | 1 | — | — | — | — | — | — | 1 |
| *Fraxinus excelsior* | — | — | — | 57 | 5 | — | — | — | — | — | — | — | 62 |
| *Polemonium(e)* | — | — | — | — | — | — | 1 | — | — | — | — | — | 1 |
| Labiatae(*e*) | — | — | — | — | — | — | 1 | — | — | — | — | — | 1 |
| *Plantago* | — | — | — | 3 | 127 | 248 | 186 | 77 | 7 | 1 | — | — | 649 |
| Chenopodiaceae | — | — | — | — | — | 1 | 11 | 12 | 3 | — | — | 1 | 28 |
| *Rheum(e)* | — | — | — | — | — | 9 | — | — | — | — | — | — | 9 |
| *Rumex* | — | — | — | — | 16 | 169 | 77 | 8 | 3 | 1 | — | — | 274 |
| *Buxus(e)* | — | — | — | — | 1 | — | — | — | — | — | — | — | 1 |
| *Mercurialis* | — | — | — | 5 | 1 | — | — | — | — | — | — | — | 6 |
| *Ulmus* | 1 | — | 206 | 673 | 16 | 2 | 2 | — | — | — | — | — | 900 |
| *Urtica* | — | — | — | — | 3 | 478 | 264 | 207 | 53 | 1 | — | — | 1006 |
| *Platanus* | — | — | — | 1 | — | — | 3 | — | — | — | — | — | 4 |
| *Betula* | — | — | — | 2099 | 258 | 13 | — | 2 | — | — | 1 | — | 2373 |
| *Alnus* | — | 4 | 135 | 24 | 4 | — | — | — | — | 1 | — | — | 168 |
| *Carpinus* | 1 | — | — | 59 | 13 | — | — | — | — | — | — | — | 73 |
| *Corylus* | 3 | 42 | 294 | 37 | 1 | 1 | 7 | 3 | — | — | — | — | 388 |
| *Fagus* | — | — | — | 3 | 60 | — | — | — | — | — | — | — | 63 |
| *Quercus* | — | — | — | — | 522 | 9 | — | — | — | — | — | — | 531 |
| *Castanea(e)* | — | — | — | — | — | 17 | 30 | 3 | — | — | — | — | 50 |
| *Juglans* | — | — | — | — | 5 | — | — | — | — | 1 | — | — | 6 |
| *Salix(e)* | — | — | 6 | 29 | 16 | 4 | 2 | — | 2 | — | — | — | 59 |
| *Populus* | — | — | 2 | 36 | — | — | — | — | — | — | — | — | 38 |
| Liliaceae(*e*) | — | — | — | — | 2 | 14 | 3 | — | — | — | — | — | 19 |
| Juncaceae | — | — | — | 7 | 12 | 8 | 16 | 2 | — | — | — | — | 45 |
| *Triglochin* | — | — | — | — | 1 | 3 | 8 | — | — | — | — | — | 12 |
| Cyperaceae | — | — | — | 1 | 27 | 3 | — | — | — | — | — | — | 31 |
| Gramineae | 5 | 3 | 3 | 33 | 104 | 4255 | 2691 | 120 | 34 | 17 | 4 | 1 | 7270 |
| *Picea* | — | — | — | — | 14 | — | — | — | — | — | — | — | 14 |
| *Pinus* | — | — | — | 2 | 68 | 15 | 5 | 1 | — | 1 | — | — | 92 |
| *Taxus* | — | — | 405 | 98 | 7 | — | 1 | — | 1 | — | — | — | 512 |
| Damaged or unexpanded | — | — | 5 | 23 | 51 | 32 | 85 | 30 | 13 | 9 | — | — | 248 |
| Unidentified | 4 | 7 | 1 | 19 | 8 | 9 | 12 | — | 8 | — | 1 | — | 69 |
| Totals | 16 | 59 | 1061 | 3226 | 1470 | 5546 | 3728 | 605 | 183 | 88 | 7 | 2 | 15991 |

* Entomophilous types are indicated by (*e*).

Fig. 3 (non-tree pollens). Notes on the various types of pollens encountered, with special reference to the local occurrence of the species or other groups concerned, are given in the next section.

## LIST OF POLLEN TYPES RECOGNIZED, WITH NOTES ON THE LOCAL OCCURRENCE OF THE SPECIES OR OTHER GROUPS CONCERNED

The total number of grains of each type caught during the year is stated in brackets after the name. Except where otherwise stated the notes refer to an area included within 1½ miles radius of the Hospital. The symbols ($A_1$, $A_2$, *Ac*, etc.), are those used in the sketch map (Fig. 1) to indicate the positions of isolated trees, etc., of the rarer species. It is not claimed that every such tree has been plotted though the whole area has been reconnoitred. The list follows the general sequence of Druce's *British Plant List*.

*Clematis Vitalba* (11). Very abundant in hedgerows.

*Thalictrum* (15). No wild species occur in the neighbourhood, but *T. aquilegifolium*, *T. glaucum* and *T. minus* var. '*adiantifolium*' are all commonly cultivated.

Other Ranunculaceae (69). The great bulk of the pollen so recorded is doubtless derived from the three common species of buttercup, all of which are abundant in the local grass fields.

*Papaver* (12). *P. Rhoeas* in gardens near Hospital.

Cruciferae (242). *Brassica arvensis* and *B. alba* were both abundant on disturbed ground immediately to the west of the Hospital in 1942, and presumably accounted for the surprising abundance of this type of pollen.

Caryophyllaceae (1). The common species occur but are nowhere specially abundant.

*Tilia* (9). Common lime (*T. vulgaris*): a common street tree in Penarth ($Ti_1$); one tree at Dinas Powis ($Ti_2$).

*Impatiens* (10). *I. glandulifera* is well established on river banks in the Cardiff district (the nearest point being Ely Bridge, 2½ miles north-west of the Hospital), though we have not seen it in the Hospital area.

*Ilex Aquifolium* (2). Woods, occasional.

*Aesculus Hippocastanum* (10). Single trees near Llandough Church ($H_1$) and Llandough House ($H_2$) respectively; half a dozen at Woodlands, Leckwith ($H_3$); common as a street tree in Penarth ($H_4$).

*Acer* (23). *A. campestre*: woods and hedgerows; common throughout the area. *A. Pseudoplatanus*: a belt of a dozen trees around the Penarth Sanatorium ($Ac_1$); three to four trees on Sully Road ($Ac_2$) and three at Dinas Powis ($Ac_3$); and elsewhere.

Leguminosae (22). The following are abundant on or near the Hospital site: *Genista tinctoria*, *Ononis repens*, *Melilotus altissima*, *Trifolium repens*, *T. dubium*, *Lotus corniculatus*, *Vicia Cracca*. The few grains observed were almost all *Lotus corniculatus*.

*Spiraea* (27). *S. Ulmaria*: abundant in damp fields below the Hospital and beside the Wrinstone Brook, as at Dinas Powis.

*Poterium Sanguisorba* (4). Quarry to north of Hospital (*Pt*); cliff tops, Penarth (2½ miles to south-west).

Other Rosaceae (55). *Crataegus monogyna* and *Prunus spinosa*, common in hedges; *Prunus* and *Pyrus* (ornamental spp.) planted outside Hospital; apples and pears, etc., in gardens.

*Ribes* (8). *R. nigrum*, *R. rubrum* and *R. Grossularia*, all common in gardens.

*Epilobium* (1). Probably *E. angustifolium*: woodland clearings.

Umbelliferae (156). *Anthriscus silvestris*, abundant in hedgerows and similar situations: the chief wave of umbelliferous pollen coincides with the flowering of this species; *Daucus Carota* and *Heracleum Sphondylium* are both very common on the site.

*Hedera Helix* (16). Common.

*Sambucus nigra* (86). Common in hedges, etc.

*Viburnum Tinus* (4). Planted on site.

Rubiaceae (3). *Galium verum*, abundant on site; *G. Aparine* in hedges.

Cichorieae (24). Several species belonging to this tribe abundant as weeds.

*Artemisia* (89). *A. vulgaris*: common on waste ground.

Other Compositae (87). Most abundant in October (Michaelmas daisies—*Aster* spp.).

Ericaceae (1). None seen or recorded in or near the area. *Calluna vulgaris* is not uncommon in the Cardiff district.

*Primula* (1). *P. vulgaris*, common.

*Fraxinus excelsior* (62). Dominant or co-dominant in almost all local woods. The smallness of the pollen catch and the shortness of the 1942 season are both remarkable.

*Polemonium* (1). Presumably from a local garden.

Labiatae (1). No species outstanding.

*Plantago* (649). *P. lanceolata*, abundant, and *P. major*, very common; the remarkable length of the flowering season is reflected in the catch.

Chenopodiaceae (28). *Chenopodium album* is very common as a weed. *Atriplex* (*Obione*) *portulacoides* and other halophilous species occur in salt-marshes fringing the Taff Estuary.

*Rheum* (9). Rhubarb commonly cultivated.

*Rumex* (274). *R. Acetosa* abundant in meadows. *R. crispus* and *obtusifolius*, both very common.

*Buxus sempervirens* (1). Planted near Hospital.

*Mercurialis perennis* (6). Woods and hedgerows, abundant.

*Ulmus* (900). *U. glabra*, frequent in woods; both it and *U. procera* frequent also in hedgerows. *U. stricta* in Llandough Churchyard.

*Urtica dioica* (1006). Weed, abundant.

*Platanus* (4). *P. acerifolia*, one tree at Cwrt yr Ala (*Pa*), and some small street trees in Penarth.

*Betula* (2373). Birches are not common in the area. Five trees (*B. alba*) at south end of Reservoir Wood ($B_1$) and an occasional tree in Coed Clwyd Gwyn ($B_2$); one small tree on site, close to and west of the operating theatre: this might have been held responsible for the high pollen counts in 1942 (especially in view of the facts that it was cut down in the winter of 1942–3 and that the 1943 birch count (Hyde & Williams, unpublished) has been relatively very small), but that at the relevant period the wind was blowing consistently in the wrong direction; single trees near Llandough-Penarth cross-roads ($B_3$) and at Dinas Powis ($B_4$); a dozen street trees in Penarth. Observations in the Cardiff district generally indicated that in 1942 birch flowered longer and more profusely than in 1943.

*Alnus glutinosa* (168). One tree at south end of Reservoir Wood ($A_1$); abundant beside the Wrinstone Brook and Michaelston-le-Pit ($A_2$) as at Dinas Powis ($A_3$).

*Carpinus Betulus* (73). Single trees (*a*) beside road ¼ mile north-west of Llandough Church ($C_1$); (*b*) at 'Woodlands', Leckwith ($C_2$); (*c*) at top of Leckwith Hill ($C_3$); (*d*) Cwrt yr Ala House (Miss E. Vachell) ($C_4$).

*Corylus Avellana* (388). Woods and hedges, abundant.

*Fagus sylvatica* (63). Two trees in Llandough village, var. *purpurea* ($F_1$); one near Cogan ($F_2$); about a dozen around Cwrt yr Ala House ($F_3$); others scattered in Cwrt yr Ala Park ($F_4$) and Hales Wood ($F_5$).

*Quercus* (531). *Q. Robur*: woods, co-dominant.

*Castanea* (50). Two trees at Cwrt yr Ala (*Cs*).

*Juglans* (6). *J. regia*: one small tree in garden adjoining Hospital site to east.

*Salix* (59). *S. caprea*: woods and hedges, common. Other species occur, lengthening the season in some years from mid-March until the end of May.

*Populus serotina* (38). Two trees at Hospital entrance ($Po_1$); twelve in Reservoir Wood ($Po_2$); one at 'Woodlands', Leckwith ($Po_3$); two on Sully road ($Po_4$).

Liliaceae (19). The grains observed agree in all respects with those of *Allium ursinum*, which occurs in the area.

Juncaceae (45). *Luzula campestris*: pastures, frequent. *Juncus 'communis'*: common in wet places.

*Triglochin* (12). Probably *T. maritimum*: in estuarine salt marsh.

Cyperaceae (31). Various Carices observed in area.

Gramineae (7270). Grass pollen is liberated in very large quantities over a large part of the area.

*Picea* (14). Single tree (*P. Abies*) at 'Woodlands', Leckwith (*Pc*).

*Pinus* (92). *P. silvestris*: several smallish trees near Cogan Station ($P_1$); 30–40 trees on high ground near east end of Coed yr Eglwys ($P_2$); two trees beside road to north of Llandough ($P_3$); two small trees in Lime Kiln Wood ($P_4$); ten trees around Cwrt yr Ala ($P_5$); half a dozen at 'Woodlands', Leckwith ($P_6$); forty small trees on Sully Road ($P_7$); twelve at Dinas Powis ($P_8$); and intermixed with deciduous trees in woods at St Andrews Major ($P_9$).

*Taxus* (512). *T. baccata*: one male tree in Michaelston-le-Pit Churchyard ($T_1$); seven near Cwrt yr Ala House ($T_2$); and others in Cwm George ($T_3$), at Dinas Powis ($T_4$) and at St Andrews Major ($T_5$).

## Relation between pollen deposition and weather conditions

The total pollen catch oscillates rather violently from day to day: this may be due in part to the undoubted lack of precision in the gravity slide method as usually practised, but some connexion also with weather conditions seems to be obvious. Meteorological data relating to the Cardiff district (maximum and minimum temperatures, bright sunshine, rainfall and wind) have therefore been carefully considered in relation to the daily pollen catch and sunshine records have also been appended in graphic form to our groups of pollen curves (Figs. 2, 3).*

* The weather observations, which have been supplied by the courtesy of the City of Cardiff Public Health Department, were made at the Meteorological Station, Penylan, which is situated at 202 ft. altitude on an open site to the north of Cardiff and about 4¼ miles roughly N.N.E. of Llandough Hospital. The various instruments are read at 9 a.m. G.M.T. The maximum temperature is attributed to the day preceding that on which the thermometer is read, the minimum to the actual day of the reading. The rainfall and the hours of bright sunshine are credited to the preceding day. The velocity of the wind is estimated visually

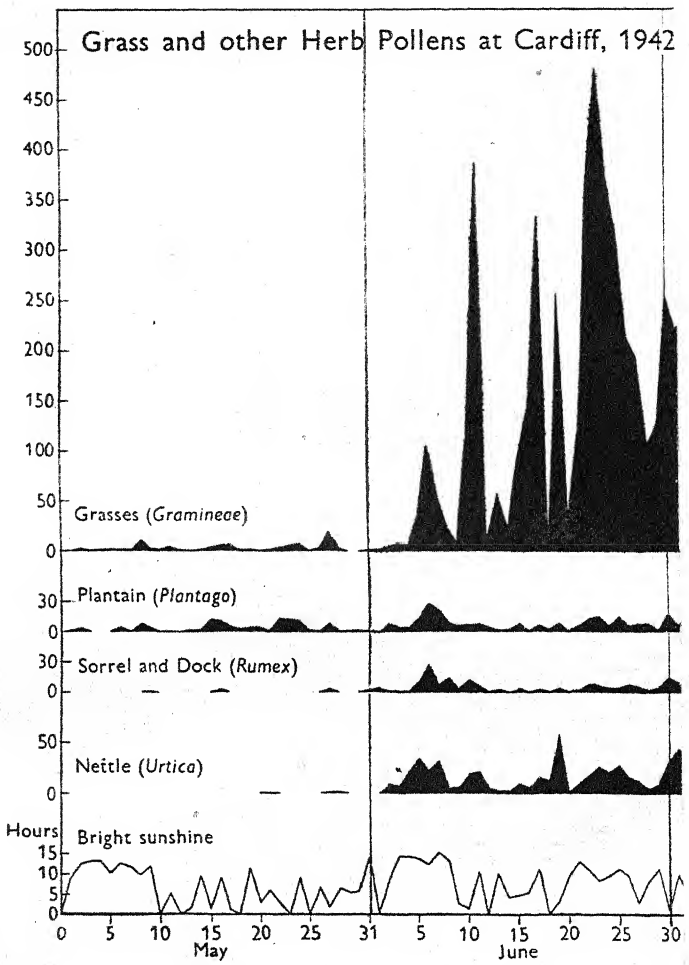
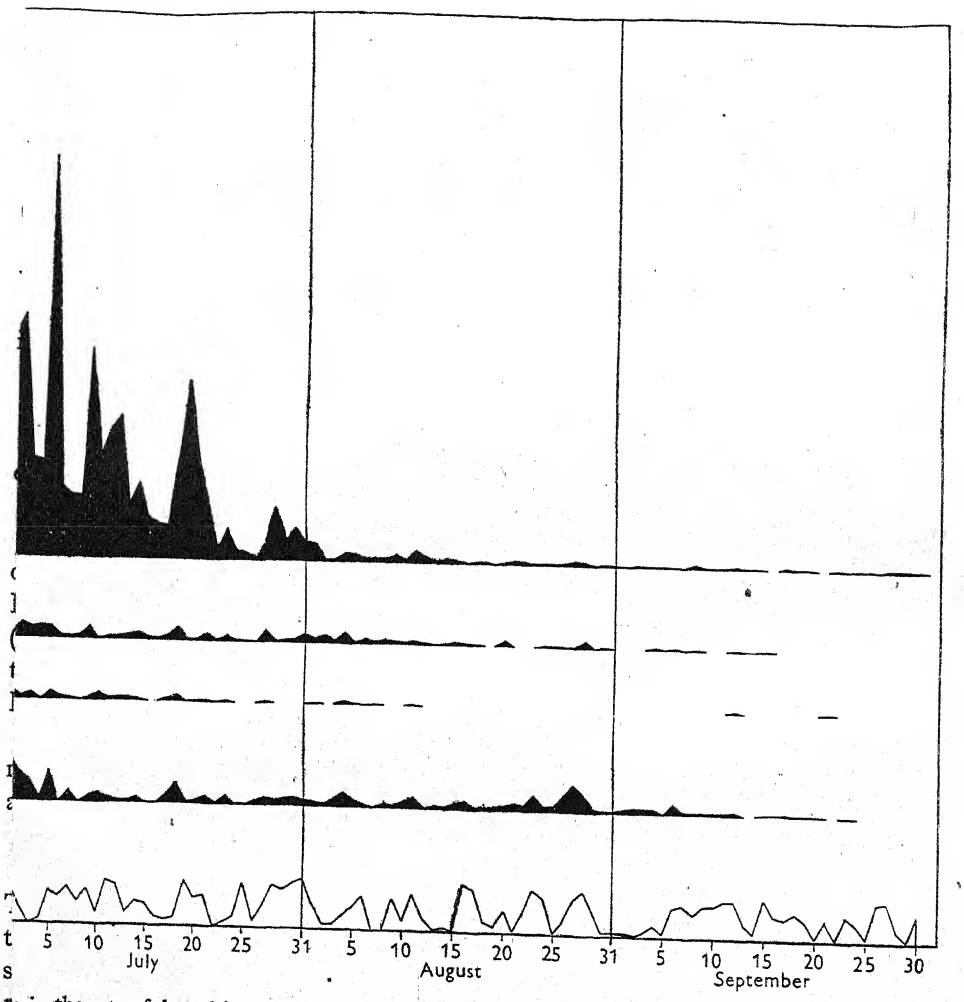



Fig. 3. Graphs showing the day-to-day variatio
of herbaceous




in in the rate of deposition of grass pollen and of the other principal types pollen at Llandough near Cardiff (1942).

It is difficult to disentangle the apparent effects of the various factors on pollen deposition, and only statistical analysis would enable this to be done completely. In what follows the more obvious apparent relationships only are pointed out.

Mild weather early in the year was followed by liberation of tree pollens: two days with a maximum of 49° F. gave 3 *Corylus* on 25 January (this species was flowering in Leckwith woods on 24 January). A recurrence of such weather in February brought this pollen up to 12 on the 13th, but a succession of frosts thereafter suppressed it again. With the beginning of March the *Corylus* curve and also the *Alnus* one became more or less continuous, though both were apparently somewhat held back by frosts. (*Alnus* had put in a few sporadic appearances during February, coincident with the early flowering of some trees of this species: it was noted in flower on 1 February at Pendoylan, 7½ miles from Llandough.) *Taxus* came in effectively on 18 March and milder weather now ushered in a series of pollen maxima, viz. *Corylus* (63) on 20th, *Alnus* (29) on 25th and *Taxus* (142) on 26th. All three pollens fell off after the dates mentioned, despite continued favourable weather. Meanwhile *Ulmus*, which had come in only a week earlier, rose to 83 on 27th and after a steep fall mounted on 1 April (a very windy day, Beaufort 6), to a maximum of 107. This pollen continued to come through in varying but decreasing quantity until the end of April: perhaps some of the oscillations in this curve are due to the fact that two or possibly three species are concerned.

*Betula* came in on 11 April following a day of 11·8 hr. of bright sunshine, and a spell of warm sunny weather brought this pollen on the 16th to a submaximum of 126. A trough in the *Betula* curve which followed coincided with a falling off in sunshine, but the curve rose again on the 23rd, and with the recurrence of favourable conditions *Betula* rose on 27 April to its maximum (387) while *Alnus* and *Taxus* about the same time experienced their final burst of activity. (Field observations on 15 April showed that while most of the alders at Dinas Powis had finished flowering, the open catkins of some still held pollen, while one tree was seen with its catkins not yet elongated.) The *Betula* curve fell more or less continuously after 27 April in spite of continued favourable weather.

*Fraxinus* pollen was deposited in almost insignificant amounts and almost all in two separate bursts during 11–14 and 24–26 April. The occurrence of east winds at these periods and west winds in between was probably fortuitous.

A late rise in the *Betula* curve toward a post-maximal peak of 37 on 9 May (coinciding with the maximum for *Fagus* (20) and *Picea* (6) on the same day), and a premaximal rise of *Quercus* to 67 on 8 May all followed on a spell of warm sunny weather: all three curves declined thereafter; *Quercus*, however, recovered and advanced to a maximum of 103 on 16 May, after which it fell steadily to zero before the end of the month. *Pinus* came in effectively toward the middle of the month and attained its maximum (23) on 27 May, a day which was unaccountably rather prolific in pollen. By the end of May the tree season had virtually ended, though *Pinus* came through, a few or only one at a time, for several weeks after.

in accordance with the specification of the Beaufort Scale for use on land. The scale extends from 0 (calm) to 12 (hurricane); the values of the numbers which appear in our records are as follows: 0, less than 1 m.p.h.; 1, 1–3 m.p.h.; 2, 4–7 m.p.h.; 3, 8–12 m.p.h.; 4, 13–18 m.p.h.; 5, 19–24 m.p.h.; 6, 25–31 m.p.h. at 33 ft. above ground. The fact that wind observations are made only once a day sets an obvious limit to their value. Unfortunately publication of our tables of pollen analyses and weather data proves impossible in war-time, but several photographic copies have been made and may be consulted on application to either of the authors.

A heat wave from 2 June onwards preceded the entry of *Urtica* and its sudden attainment of a pre-maximum of 34 on 5 June. *Plantago*, which had been deposited steadily though not abundantly throughout May, went up to its maximum (28) on 6 June. Both these pollens continued to come through during the month, and nettle rose to 58 on 19 June, though *Plantago* seldom exceeded 15. *Rumex* pollen which had been collected occasionally in small numbers in May also rose rapidly to 25 (*Rumex Acetosa*) on 6 June after which it declined, rising again later in the month with the flowering of the common species of dock. But the feature of the month was the grass curve. This type of pollen, which had been caught in small numbers (up to 20) during May, rose suddenly with the heat wave of 2–5 June to 105 on 6 June: the falling off on 7–8 June, in spite of the continued sunshine, may have been related to the 20° drop in temperature. From then on until late in the month weather conditions and the deposition of grass pollen went hand in hand. Thus a low catch on 9 June corresponded with a break in the sunny weather; a sunny day on 11 June (maximum temperature only 61°) brought a pre-maximum of 380, and similar conditions (11 hr. bright sunshine and 65° F. max.) on 17th gave 333; the following (sunless) day gave only 7 grains; and the rapid rise to the season's maximum (482 on 23rd) came during a period of hot weather with long days of bright sunshine. After this date there was still a general correspondence between bright sunshine and grass pollen deposit but the (apparent) response evoked by long sunny days became less and less, until in spite of a spell of fine warm weather during 28–31 July the grass catch fell rapidly to a low figure.

Plantain and nettle continued to be deposited in some quantity throughout July, but *Rumex* fell almost to nothing by 23rd.

By 1 August the total daily catch, which had fallen to the neighbourhood of 40, was made up preponderantly of herbaceous dicotyledons, viz. *Plantago*, *Urtica* and one newcomer, viz. *Artemisia*, which reached its highest figure (12) on the 20th. The curves representing these species show that they all declined in numbers at this period, only experiencing a sharp temporary rise coincident with a short heat wave during 27–29 August. Plantain virtually died away at the end of the month and *Artemisia* a week later, but nettle continued until after mid-September. Grasses trickled through even in October.

To sum up on the influence of weather conditions: In spring, warm spells bring about the liberation of pollens in those species which presumably have already reached the flowering stage: conversely frosts cause its temporary cessation. It would appear however that once general liberation has been induced by favourable conditions the onset of only moderately unfavourable conditions does not greatly affect the situation. In the early summer hot sunny weather is evidently conducive to the presence of large quantities of grass pollen in the air. We have made additional observations on this point, which will be published separately.

Our results do not appear to show any marked effect of rainfall *per se* on the quantity of pollen in the air.

There is some evidence that, other things being equal, windy days bring most pollen to the apparatus.

## DISCUSSION

It would be premature to draw any far-reaching conclusions from the results obtained at one station during a single season; but the following points appear to deserve mention.

### (*a*) *Origin of pollen caught at Llandough Hospital*

We set out with an open mind on the subject of the probable distance of origin of the bulk of the pollen precipitated at a particular place. What we had read in the literature led us to expect that distant influences might be important at least at times. However, comparison between our monthly tables and the flora of the neighbourhood shows that the occurrence and frequency of almost every pollen type recorded might be accounted for locally: no type occurred which could not have originated within a few miles at the most. We think that under ordinary atmospheric conditions a considerable proportion of the pollen caught in our apparatus was of 'local' origin in Bertsch's sense (0–500 m.) (Bertsch, 1935); 'near transport' (500–1000 m.) would account for all but a very few types, for which 'wide transport' (1–10 km.) would have to be invoked, whilst none would necessitate 'distant transport' (over 10 km.). No doubt in some circumstances distant transport might be an important factor but our slides provide no certain evidence of its occurrence. We do not agree with Bertsch that a grain occurring out of the local season of the plant concerned is necessarily evidence of distant transport: it may have settled first on the ground or on a tree and then later have been dislodged by the wind. We have observed a number of such grains: they are almost always accompanied by dirt.

### (*b*) *Entomophilous species as airborne pollens*

As was to be expected pollens of anemophilous species greatly preponderated in the catch, the actual figures for identified grains being 14,649 (93·5 %) anemophilous against 1024 (6·5 %) entomophilous pollens. The latter group is not, however, negligible and further analysis based on Knuth's pollination types (Knuth, 1906) is of interest. This shows (Table 2) that except for Compositae, only a very small proportion of the ento-

Table 2. *Pollens derived from entomophilous flowers grouped according to Knuth's types of pollination mechanism*

| Knuth's types | Types of pollen | | Totals | % |
|---|---|---|---|---|
| Nectar partly concealed, type **EC** | Ranunculaceae (except *Clematis* and *Thalictrum*) | 69 | | |
| | Cruciferae | 242 | | |
| | Rosaceae (most) | 55 | | |
| | *Rheum* | 13 | | |
| | *Salix* | 59 | 438 | 43 |
| Nectar freely exposed, type **E** | *Tilia* | 9 | | |
| | *Ilex* | 2 | | |
| | *Acer* | 23 | | |
| | Umbelliferae | 156 | | |
| | *Hedera* | 16 | | |
| | *Buxus* | 1 | 207 | 20 |
| Pollen flowers, type **Po** | *Clematis* | 11 | | |
| | *Papaver* | 12 | | |
| | *Spiraea* | 27 | | |
| | *Sambucus* | 86 | | |
| | *Castanea* | 50 | 186 | 18·0 |
| Social flowers, type **S** | Compositae (other than *Artemisia*) | | 111 | 11·0 |
| Other concealed types | | | 72 | 7·0 |
| Indeterminate | | | 10 | 1·0 |
| | | | 1024 | 100 % |

mophilous grains caught are derived from flowers having fully concealed nectar or more elaborate floral mechanisms: the great majority belong to flowers with exposed (Knuth's type **E**) or partly concealed (**EC**) nectar or to pollen flowers (**Po**).

### (*c*) *The tree-pollen spectrum*

In order to facilitate comparison between our analyses as a whole and pollen analyses of peat we have extracted the figures relating to genera and species commonly occurring in British peats and set them out in the customary order, together with percentages of 'Total tree pollen' as usually understood in this connexion (Table 3). The most notable feature is the great preponderance of birch, which however we think may be abnormal: further comment is therefore deferred until the results of the wider survey of 1943 are available.

Table 3. *The tree-pollen spectrum*

| | Pine | Birch | Elm | Oak | Lime | Alder |
|---|---|---|---|---|---|---|
| Numbers of grains caught during year | 92 | 2373 | 900 | 531 | 9 | 168 |
| Percentages of 'Total tree pollen' | 2·0 | 56·0 | 21·5 | 13·0 | (0·2) | 4·0 |

| | Beech | Hornbeam | 'Total tree pollen' | Hazel | Willow |
|---|---|---|---|---|---|
| Numbers of grains caught during year | 63 | 73 | 4209 | 388 | 59 |
| Percentages of 'Total tree pollen' | 1·5 | 2·0 | 100·0 | 9·0 | 1·5 |

### (*d*) *The possible significance of the pollen catch in relation to allergic disease*

Before the middle of March and after the end of August pollen was either completely absent from the air or if present its atmospheric concentration was too small to give rise to allergic manifestations.

During the spring several tree pollens were at various times present in high concentration, the most important from the present point of view being elm, birch and oak.

Grass pollens were encountered in very high concentration as was to be expected during a period which extended from about the first week of June until the third week of July. It would appear from the observations here recorded that the grass-pollen concentration and consequently its potential danger to health varied very greatly within this period: further experiments on this point have been made and will be published separately.

*Plantago* and *Rumex* pollen have been regarded as being possible causes of allergic disease in this country; it would appear, however, from our curves that neither was present in sufficient quantity at any time to cause any ill effect. *Urtica* pollen seems not to have been recorded previously anywhere in such quantities as we have encountered: its clinical significance is unknown.

The only pollen to appear exclusively and in some quantity in the late summer is that of the genus *Artemisia*. In view of the importance attributed to this genus and to other anemophilous Compositae in America and of the fact that one species at least (*A. vulgaris*) sometimes dominates the vegetation of disturbed areas we think that this type of pollen may perhaps be of some significance.

It is evident that further observations must be made over a wide area before any general conclusions can be drawn under this heading.

## Summary

1. A year's day-to-day census of atmospheric pollen, carried out by the gravity method and believed to be the first such detailed investigation to be made in Great Britain, and in fact in Europe, is described.

2. Anemophilous pollens formed 93·5 % of the grains identified and entomophilous pollens 6·5 %.

3. The effective pollen season (in a botanical sense) lasted from early March until late September; it presented three phases: (*a*) trees, (*b*) grasses, (*c*) dicotyledonous herbs, the last named being relatively unimportant.

4. The relations of the catch to local sources and local weather influences are considered.



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# ON THE OXIDATIVE DECOMPOSITION OF HEXOSE-DIPHOSPHATE BY BARLEY

## THE ROLE OF ASCORBIC ACID

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(With 3 figures in the text)

In previous papers, summarized by James & Bunting (1941), it has been shown that the degradation of sugars in barley proceeds by way of phosphorylation to hexose-diphosphate, oxidation to phosphoglycerate and dephosphorylation to pyruvic acid. The ascorbic acid system has been shown to be an active oxidizer in barley (James & Cragg, 1943); and a possible role for it as producer of phosphoglycerate in the preceding system is elaborated in the present paper.

### Material

Barley var. Plumage Archer was used throughout. Seedlings were raised on water for 7–14 days in a warm greenhouse or dark room. Except where otherwise stated, a clear barley juice was prepared from their shoots by the method described by James & Cragg.

*Hexosediphosphate.* Aliquots of a preparation of barium hexosediphosphate were weighed out for each experiment as required and dissolved in dilute HCl. The barium was precipitated by cautious additions of sodium sulphate and centrifuged down. The supernatant solution was decanted, made up nearly to volume, and then adjusted to *p*H 6 (the approximate acidity of the sap), with a minimal quantity of sodium hydroxide. The indicator used was brom thymol blue (incubation experiments) or litmus (manometric experiments). In one or two of the manometric experiments the natural buffers of the sap were reinforced with phosphate buffer *p*H 6, but we were unable to observe that it made any significant difference.

Analysis of a solution so prepared gave the results shown in Table 1.

Table 1. *Hydrolysis of hexosediphosphate by N* $H_2SO_4$ *at* 100° C.

| | |
|---|---|
| Inorganic phosphate | $P_i$ = 13·2 % total phosphate |
| Released by 3 hr. acid hydrolysis | $P_{180}$ = 53·0 % total phosphate |
| Unhydrolysed | $P_u$ = 33·8 % total phosphate |

According to Lohmann (1928) hexose-6-phosphate loses 22·7 % of its phosphate during 3 hr. acid hydrolysis (*N* $H_2SO_4$ at 100° C.) in a sealed glass bulb, while 94 % of the second phosphate radicle of hexosediphosphate is released. With pure hexose-diphosphate the ratio $P_u/P_{180}$ to be expected would therefore be 41·6/58·4 = 0·71. The corresponding ratio for our preparation is 0·64, and there is also present some inorganic phosphate as impurity. Hexose-6-phosphates appear to be excluded.

The barley saps used in the following experiments were found to have a drastic effect on hydrolysis at 100° C. Samples of the same preparation of hexosediphosphate were

dissolved and added to aliquots of sap. The sap was immediately fixed with cold trichloracetic acid and phosphate estimations were carried out as before. An aliquot of sap without addition of hexosediphosphate was examined at the same time and the control values so obtained deducted from those of the mixture to obtain the hexosediphosphate values in presence of sap. The results of two such experiments are compared with those for pure solutions in Table 2.

Table 2

| | % acid soluble phosphate | | |
|---|---|---|---|
| | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| Hexosediphosphate (Lohmann) | 58·4 | 41·6 | 0·71 |
| Hexosediphosphate, our preparation | 53·0 | 33·8 | 0·64 |
| Hexosediphosphate + barley sap (i) | 68·8 | 16·5 | 0·24 |
| Hexosediphosphate + barley sap (ii) | 86·8 | 5·5 | 0·06 |
| Hexosediphosphate + barley sap (iii) | 76·4 | 11·5 | 0·15 |

There is an increased error in the estimation in the presence of sap since each result has to be the difference between two estimations—with and without the added hexosediphosphate. It is, however, clear that the sap reduces the $P_u/P_{180}$ ratio of hexosediphosphate very markedly; in fact the greater part of the phosphate linkages (about 6/7) are hydrolysed when it is present. This does not apply to the $P_u$ fraction of the sap itself, which is mainly due to hexose-6-phosphates (see Heard, 1943).

## Methods

*The estimation of phosphate fractions* was carried out exactly as described by Heard (1943). In view of the proved effect of the saps on the rate of acid hydrolysis, the $P_{180}$ (3 hr.) and $P_u$ (unhydrolysable) fractions could clearly not be given their usual quantitative significance. We have made no attempt to use the data in this way. A large reduction of the $P_{180}$ fraction we have regarded as indicating a loss of hexosediphosphate and a considerable rise of the $P_u/P_{180}$ ratio as indicating a transfer of phosphate from hexosediphosphate to unhydrolysable combinations, hexosemonophosphates or phosphoglycerates. Owing to the simultaneous hydrolysis of hexosephosphates there may be an accumulation of phosphoglycerate without a rise of the $P_u$ value; but it may still be revealed by a rise of the $P_u/P_{180}$ ratio.

*The estimation of oxygen consumption* was carried out manometrically at 30° C. in Barcroft manometers with Dickens-Simer flasks, with baryta in the annulus to absorb carbon dioxide. Total carbon dioxide output was determined at the end of the experiment by tipping in HCl from the side tube. Hexosediphosphate, ascorbic acid and other desired solutions were put into the main compartment of the flask and the sap to be used in a separate flask by itself. All were then brought into oxygen equilibrium by partial exhaustion and refilling with oxygen passed through soda lime, baryta and water. This was repeated four times and the manometers then shaken for 15 min. while still open to the oxygen supply. Aliquots of sap were then transferred from the separate flask into each of the experimental flasks, by a pipette maintained at the temperature of the bath. This was done as rapidly as was consistent with accuracy. The flasks were immediately stoppered, lowered into position, their gas taps closed and shaking begun.

The experiment was timed from the moment of switching on the shaker. This method of introducing the sap was not entirely satisfactory, and occasionally there was an initial lag in oxygen uptake due probably to an incomplete mixing of the sap and reagents. Experiments in which this was pronounced were discarded.

## RESULTS

*Conversion of hexosediphosphate to phosphoglycerate.* Two aliquots of cleared sap, each of 7 ml., were pipetted into two conical flasks. 2 ml. hexosediphosphate in glass-distilled water were added to each. To one was further added 1 ml. sodium fluoride to give a final concentration of $M/50$ and 0·03 g. solid thymol. The flask was then plugged with cotton-wool and incubated at 30° C. for 40 hr. To the other flask was added 1 ml. glass-distilled water. The contents of this second flask were then fixed immediately with trichloracetic acid, and phosphate determinations carried out in the usual way. The incubated digest was similarly treated at the end of the 40 hr.

Table 3

| Solution in ml. | | | | Treatment | $P$ in $\gamma$/ml. sap | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sap | HdP | NaF | GDW | | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| 7 | 2 | — | 1 | Fixed immediately | 598 | 183 | 254 | 161 | 0·63 |
| 7 | 2 | 1 | — | 30° C. for 40 hr. | 549 | 199 | 114 | 236 | 2·08 |

Concentrations in digests: HdP = 0·22 %; NaF = $M/50$.

The results show a large reduction of $P_{180}$, a small increase of inorganic phosphate ($P_i$) and a large increase of unhydrolysable phosphate ($P_u$) leading to a large increase in the $P_u/P_{180}$. These results suggest that there was a vigorous decomposition of the added hexosediphosphate and that it was converted largely to phosphoglycerate. The identification of the unhydrolysable ester as phosphoglycerate is rendered all the more probable by the presence of NaF. NaF, at the concentrations employed, does not inhibit hydrolyses but does inhibit the conversion of phosphoglycerate to phosphopyruvate. With a single exception, digests without NaF (see Table 9) showed no increase of $P_u$ or of the $P_u/P_{180}$ ratio, though inorganic phosphate continued to be hydrolysed off.

Confirmation of the formation of phosphoglycerate was sought by a solubility method, phosphoglycerate being markedly less soluble than the hexosediphosphate.

Aliquots were taken from two digests with and without incubation. They were submitted to a 3 hr. hydrolysis to remove residual hexosediphosphate and other hydrolysable phosphates. They then contained inorganic orthophosphate and mixed unhydrolysable esters, mainly hexosemonophosphate and phosphoglycerate. The solutions were adjusted to *p*H 3 and diluted with twice their volume of alcohol. On addition of barium, inorganic phosphate and phosphoglycerates are precipitated as acid salts and hexosemonophosphates left in solution. Copious precipitates were formed by this treatment in both solutions. These precipitates were removed, redissolved in dilute HCl, neutralized with NaOH and made up to suitable volume. Aliquots were taken for estimation of inorganic ($P_i$) and total ($P_T$) phosphate, the difference in this case representing unhydrolysable and precipitable phosphate, i.e. phosphoglycerate. In the unincubated mixture inorganic phosphate accounted for practically the whole of the phosphate precipitated by barium

at *p*H 3; but in the digest after incubation it accounted for only a small proportion of it, leaving a large difference attributable to phosphoglycerate. Precipitation from these saps was not quantitative, however, and duplicate analyses showed wide differences, so that it was not possible by this method to estimate the actual amount of phosphoglycerate formed.

*Oxidation in the presence of ascorbic acid.* Five aliquots of cleared sap, each of 4 ml. volume, were pipetted into conical flasks. One lot was fixed immediately by the addition of trichloracetic acid to a final strength of 10%. A second aliquot was fixed immediately after the addition of a measured quantity of hexosediphosphate. The other three flasks received additions of hexosediphosphate, ascorbic acid and sodium fluoride, as shown in Table 2. All solutions were made up in glass-distilled water and the digest in each flask adjusted to 6 ml. with glass-distilled water also. Solid thymol (approximately 3 parts to 1000 by weight) was added to each flask which was plugged tightly with cotton-wool and incubated at 30° C. for 24 hr. All the digests were then fixed with trichloracetic acid and the phosphate fractions analysed in the usual way.

Table 4

| Solution in ml. | | | | | Treatment | *P* in γ/ml. sap | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sap | HdP | NaF | AA | GDW | | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| 4·0 | — | — | — | 2·0 | Fixed immediately | 350 | 240 | 72 | 38 | 0·53 |
| 4·0 | — | 0·4 | — | 1·6 | 30° C. for 24 hr. | 304 | 223 | 53 | 28 | 0·53 |
| 4·0 | 1·0 | — | — | 1·0 | Fixed immediately | 552 | 256 | 247 | 49 | 0·20 |
| 4·0 | 1·0 | 0·4 | — | 0·6 | 30° C. for 24 hr. | 547 | 395 | 97 | 55 | 0·57 |
| 4·0 | 1·0 | 0·4 | 0·6 | — | 30° C. for 24 hr. | 538 | 408 | 46 | 84 | 1·82 |

Concentrations in digests: HdP = 0·2%; NaF = $M/75$; ascorbic acid = 0·22%.

These results again show a transfer of phosphate from $P_{180}$ to $P_i$ and $P_u$ and a large increase of the $P_u/P_{180}$ ratio during incubation. The relatively large increase of inorganic phosphate ($P_i$) suggests that a good deal of the added hexosediphosphate was hydrolysed by phosphatases to free phosphate and sugars with possibly some hexosemonophosphate as intermediate. If the values for the sap are deducted from the corresponding values after addition of hexosediphosphate the changes in the organic fractions, which may then be regarded as relating solely to the added hexosediphosphate, become even more striking. The rise of $P_u/P_{180}$ agrees with that in the previous experiment (Table 3);

Table 5

| | *P* in γ/ml. sap | | | | |
|---|---|---|---|---|---|
| | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| Hexosediphosphate before incubation | 202 | 16 | 175 | 11 | 0·06 |
| Hexosediphosphate after incubation | 243 | 172 | 44 | 27 | 0·61 |
| Hexosediphosphate after incubation with ascorbic acid | 234 | 185 | −7 | 56 | ∞ |

but here we have also a new result, viz. that the rise is much increased by the presence of ascorbic acid in the digest. It is also noteworthy that the ascorbic acid has but little effect on the release of inorganic phosphate. It seems clear, therefore, that the increase in unhydrolysable phosphate brought about by ascorbic acid must be due to its catalysing

an oxidation to phosphoglycerate. An alternative possibility of its catalysing a reduction to phosphoglycerol (also 'unhydrolysable') is ruled out by the oxygen measurements to be presented later (p. 68).

It was observed that this action of ascorbic acid did not occur if the reagents and digests were made up with water from the large laboratory still instead of from an all-glass one. A parallel experiment using ordinary distilled water gave the following results:

Table 6

| Solution in ml. | | | | Water | Treatment | $P$ in γ/ml. sap | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sap | HdP | NaF | AA | | | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| 2 | 0·5 | — | — | 0·5 | Fixed immediately | 522 | 279 | 184 | 59 | 0·32 |
| 2 | 0·5 | 0·2 | — | 0·3 | 30°C. for 24 hr. | 489 | 350 | 119 | 20 | 0·17 |
| 2 | 0·5 | 0·2 | 0·3 | — | 30°C. for 24 hr. | 529 | 347 | 133 | 49 | 0·37 |

Concentrations in digests: HdP = 0·2 %; NaF = $M/75$; ascorbic acid = 0·22 %.

In this experiment there was no marked change in $P_u/P_{180}$ and the phosphate lost by $P_{180}$ was all transferred to $P_i$. $P_u$, in sharp contrast with the previous experiments, showed a falling off. A second experiment was carried out and gave similar results. The most significant impurity likely to be present in the ordinary distilled water would be traces of copper. Enough is present to bring about the irreversible oxidation of ascorbic acid on standing at room temperature. It was therefore sought to discover whether the results obtained with ordinary distilled water were reproduced by glass-distilled water to which a trace of copper was added. Parallel digests were made up

Table 7

| Solution in ml. | | | | | | Treatment | $P$ in γ/ml. sap | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sap | HdP | NaF | AA | $CuSO_4$ | GDW | | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| 7 | 1·5 | — | — | 0·5 | 2·0 | Fixed immediately | 514 | 289 | 117 | 108 | 0·92 |
| 7 | 1·5 | 1·0 | — | 0·5 | 1·0 | 30°C. for 24 hr. | 504 | 361 | 59 | 84 | 1·42 |
| 7 | 1·5 | 1·0 | 1·0 | 0·5 | — | 30°C. for 24 hr. | 496 | 365 | 68 | 63 | 0·93 |
| 7 | 1·5 | — | — | — | 2·5 | Fixed immediately | 496 | 283 | 128 | 85 | 0·66 |
| 7 | 1·5 | 1·0 | — | — | 1·5 | 30°C. for 24 hr. | 492 | 330 | 81 | 81 | 1·00 |
| 7 | 1·5 | 1·0 | 1·0 | — | 0·5 | 30°C. for 24 hr. | 500 | 348 | 64 | 88 | 1·37 |

Concentrations in digests: HdP = 0·16 %; NaF = $M/55$; AA = 0·20 %; $CuSO_4$ = $M/2200$.

with and without addition of $CuSO_4$ to a concentration of $M/2200$. The digests without Cu differ from the previous experiments in not showing an increase of $P_u$; but there is a loss of $P_{180}$ and a rise of $P_u/P_{180}$ and this is again greatest in the presence of ascorbic acid. The digests with copper resemble those with normal (Cu) distilled water in showing a loss of 'unhydrolysable' phosphate and no rise of $P_u/P_{180}$ in the presence of ascorbic acid. The rise in the absence of ascorbic acid depends on an anomalous $P_{180}$ value and has not been confirmed. In all digests there is the usual increase of inorganic phosphate.

The concentration of NaF used in these experiments is not such as would inhibit the enzymic decomposition of phosphoglycerate entirely; though it might be expected to retard it. The results observed may, therefore, be best explained by supposing that,

in the presence of ascorbic acid, phosphoglycerate formation is accelerated. The addition of copper leads to the destruction of the ascorbic acid so that phosphoglycerate formation becomes slower than its decomposition.

*The formation of triosephosphate.* A further analysis showed that other changes not revealed by the foregoing experiments had also been taking place within the $P_{180}$ fraction. The triosephosphates (phosphoglyceraldehyde and dihydroxyacetone phosphate) are completely hydrolysed by 1 hr. acid hydrolysis and would, therefore, be included in $P_{180}$. They are highly unstable in an alkaline medium and their phosphate ($P_a$) is completely released at room temperature after 20 min. in $N$ NaOH (1 vol. sap. + 1 vol. $2N$ NaOH). They were not initially present in considerable quantity in our extracted saps. Examination of aliquots from the digests of the preceding experiment showed, however, that they were formed during the incubation in the presence of NaF and that the breakdown of hexosediphosphate was correspondingly greater than appeared from the total $P_{180}$ fraction.

Further experiments were therefore performed using methods likely to inhibit the oxidation of any triosephosphate formed. Incubations of sap were carried out in the usual way; but the NaF was replaced by cyanide, which forms cyanhydrins with triosephosphates, and bisulphite which forms the usual carbonyl bisulphite. In addition to these two fixatives, iodoacetate, which inhibits oxidation of triosephosphate by coenzyme I, was also used. The analyses of alkali-labile phosphate in all these experiments are collected in Table 8.

Table 8. *Increase in alkali-labile phosphate after* 24 *hr. at* 30° C.

| Digest (sap 2/3, reagent solutions 1/3) | $P_a$ in $\gamma$/ml. sap |
|---|---|
| Sap + HdP + $M$/55 NaF | 15 |
| Sap + HdP + $M$/55 NaF | 16 |
| Sap + HdP + $M$/55 NaF + $M$/2200 $CuSO_4$ | 20 |
| Sap + HdP + $M$/55 NaF + $M$/2200 $CuSO_4$ | 15 |
| Sap + HdP + $M$/686 iodo-acetate | 18 |
| Sap + HdP + $M$/100 HCN (KCN, *p*H 5) | 44 |
| Sap + HdP + $M$/100 HCN (KCN, *p*H 5) | 40 |
| Sap + HdP + $M$/10 $NaHSO_3$ | 43 |

In every case there was a formation of alkali-labile phosphate during the incubation. The enzyme inhibitors, NaF and iodoacetate, each had about the same effect. The two aldehyde fixatives, HCN and bisulphite, were also equally effective, and much more so than the inhibitors. Herbert, Gordon, Subrahmanyan & Green (1940) have shown that highly purified zymohexase loses its power to split hexosediphosphate to triosephosphate, in the presence of $M$/50,000 $CuSO_4$. In our complete saps this inhibition is prevented and the formation of alkali-labile phosphate went on at $M$/2200.

The usual phosphate fractions were also determined in these experiments with the results shown in Table 9. As might be expected there is no evidence here of an appreciable increase of unhydrolysable phosphate as phosphoglycerate, the reaction having been blocked at an earlier stage. Breakdown of hexosediphosphate occurred in each case. To make this clear it is necessary to deduct the $P_a$ value from the $P_{180}$ values; thus in the presence of sodium bisulphite the corrected $P_{180}$ was $190-0=190$ before incubation and $193-43=150$ after incubation.

Table 9

| Solution in ml. | | | | | Treatment | $P$ in $\gamma$/ml. sap | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sap | HdP | KCN | Iodo-acetate | GDW | | $P_T$ | $P_i$ | $P_{180}$ | $P_u$ | $P_u/P_{180}$ |
| 4 | 2 | — | — | 0·86 | Fixed immediately | 903 | 398 | 363 | 142 | 0·39 |
| 4 | 2 | — | 0·86 | — | 30° C. for 24 hr. | 847 | 629 | 167 | 51 | 0·31 |
| 4 | 2 | 0·86 | — | — | *p*H 5 for 24 hr. 30° C. | 935 | 496 | 264 | 175 | 0·66 |
| Sap | HdP | KCN | $NaHSO_3$ | GDW | | | | | | |
| 5 | 2 | — | — | 1 | Fixed immediately | 835 | 617 | 190 | 28 | 0·15 |
| 5 | 2 | — | 1 | — | 30° C. for 24 hr. | 761 | 535 | 193 | 33 | 0·17 |

Concentrations in digests: HdP ~ 0·2 %; KCN = $M$/100; $NaHSO_3$ = $M$/10; iodo-acetate = $M$/686.

$O_2$ *uptake in the presence of ascorbic acid and hexosediphosphate.* A sap was extracted from young barley seedlings in the usual way. It was allowed to stand in the refrigerator until required and the cloudy precipitate which formed was centrifuged down. On being examined manometrically (p. 63) this sap, by itself, showed no uptake of $O_2$, and on addition of 2 mg. ascorbic acid/ml. sap it showed an $O_2$ uptake of 0·75 cu.mm. $O_2$/ml. sap/min. It thus contained an ascorbic oxidase with similar characteristics to that found previously (James & Cragg, 1943). The behaviour of this system towards hexosediphosphate was examined with an aliquot of the same hexosediphosphate preparation as was used in the analytical experiments. The time curves of $O_2$ uptake are collected in Fig. 1. Hexosediphosphate alone caused slight uptake of $O_2$, but in the

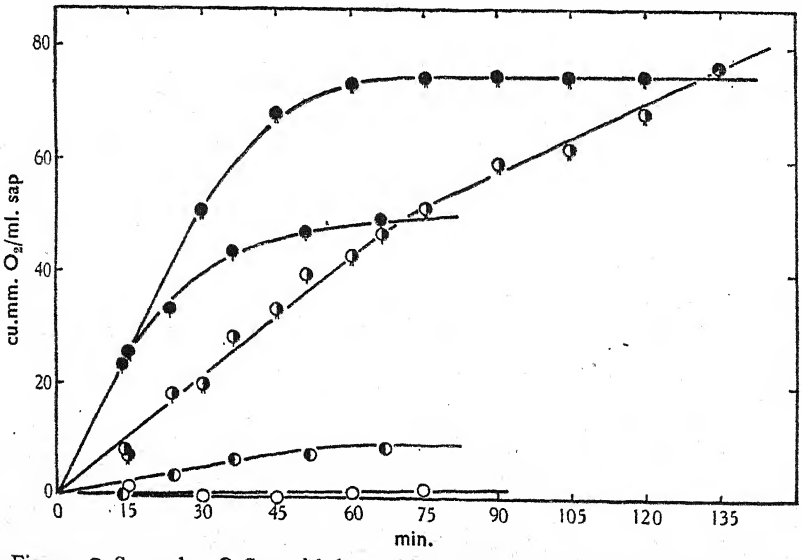



Fig. 1. ○ Sap only. ◐ Sap with hexosediphosphate. ◑ Sap with ascorbic acid, two experiments. ● Sap with ascorbic acid and hexosediphosphate.

presence of ascorbic acid it caused a large increase. Rates, read from the initial slopes of the curves, are:

| | cu.mm. $O_2$/ml. sap/min. |
|---|---|
| Sap only | 0 |
| Sap + hexosediphosphate | 0·15 |
| Sap + ascorbic acid | 0·72 |
| | 0·87 |
| Sap + HdP + ascorbic acid | 1·51 |

The rate of $O_2$ uptake was greater with the mixture than the sum of the two rates when HdP and ascorbic acid were added separately; in other words, was not merely due to the additive effect of two independent processes, but to the oxidation of hexosediphosphate via ascorbic acid. In an experiment with other aliquots of the same sap, the same rate of oxidation of ascorbic acid was observed. The oxidation of hexosediphosphate (in the presence of ascorbic acid) was initially the same as before, but continued longer. The oxidation of ascorbic acid alone does not lead to $CO_2$ formation, whereas in the presence of hexosediphosphate some $CO_2$ is produced. As no baryta was used in the latter experiment, it follows that the rate of oxidation of hexosediphosphate via ascorbic acid (but not of ascorbic acid alone) is underestimated, and the differences even greater than shown.

A further feature of the results is also noticeable. The rapid oxygen uptake in the presence of both hexosediphosphate and ascorbic acid is not continued indefinitely, but falls off and comes to a standstill after about 60 min. Oxidation continues longer with ascorbic acid only, and eventually as much $O_2$ is consumed as with the mixture. Even so, $O_2$ consumption does not proceed to a quantity equivalent to the total ascorbic acid added. It has been shown that loss of ascorbic acid is always greater than absorption of $O_2$ (James & Cragg, 1943). This is attributable to oxidizing substances present in the sap and it is not surprising to find that their quantity is markedly increased by hexosediphosphate addition. This secondary effect was shown by nearly all the saps examined.

It was found that the method of clearing the sap had considerable effect on its capacity to oxidize hexosediphosphate without addition of ascorbic acid. In crude saps it was quite considerable. Table 10 shows the rate of oxygen uptake by a series of preparations. Sap II, before precipitation, also showed a spontaneous uptake of $O_2 \sim 1{\cdot}9$ cu.mm. $O_2$/ml. sap/min., without the addition of any substrate at all. Although crude saps usually showed a faster oxygen absorption with hexosediphosphate + ascorbic acid than with ascorbic acid alone, the rate of the mixture did not exceed the sum when the two components were taken separately. It could not, therefore, be said with certainty that the hexosediphosphate was being oxidized in such saps via ascorbic acid.

Table 10. *Oxygen uptake by barley saps + hexosediphosphate over first* 30 *min.*

| Sap preparation | cu.mm. $O_2$/ml. sap/min. | Method of clearing |
|---|---|---|
| V | 1·8 | Cloudy sap used immediately |
| IV | 2·9 | Kept 2 days at 0° C., centrifuged |
| II | 3·0 | Kept 1 day at 0° C., centrifuged |
| II | 0·0 | Ba precipitate removed |
| I | 0·15 | Kept 10 days at 0° C., centrifuged |

Sap II, after Ba precipitation, was used for a further hexosediphosphate + ascorbic acid experiment with the results shown in Fig. 2. In this clarified sap there was no oxidation of hexosediphosphate alone. The initial rate of $O_2$ absorption in the presence of hexosediphosphate + ascorbic acid was faster than with ascorbic acid alone; and the curves met again in just under 1 hr. The initial slopes indicate the following rates:

| | cu.mm. $O_2$/ml. sap/min. |
|---|---|
| Sap + hexosediphosphate | 0·0 |
| Sap + ascorbic acid | 5·3 |
| Sap + hexosediphosphate + ascorbic acid | 9·3 |

The sap, when compared with the one cleared by standing, was, therefore, about seven times as active in oxidizing ascorbic acid and about six times as active in oxidizing hexosediphosphate via ascorbic acid. Apart from the absolute rates, the behaviour of the preparations was very similar. Other results of the same kind with another barium-cleared sap are shown in Fig. 3.

*Coenzyme I.* The experiments described on p. 67 show that triosephosphate is formed and is, therefore, the probable substance oxidized. In yeast and animal tissues co-enzyme I acts as H transporter in this oxidation; and it was, therefore, of interest to discover whether the $H_2$ of dihydro-coenzyme I could be removed through our ascorbic acid system. Through the generosity of Prof. R. A. Peters, who provided us with a

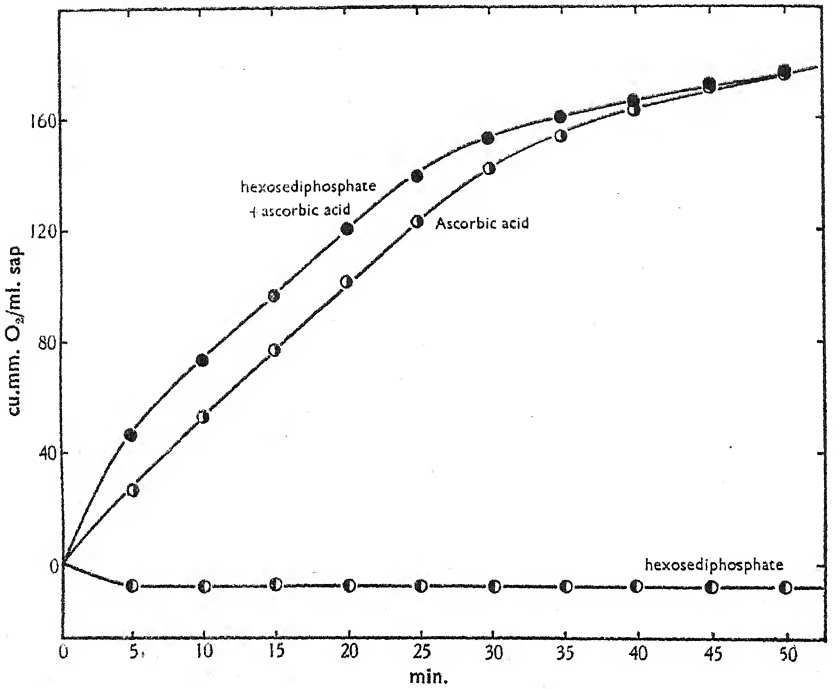



Fig. 2. ◐ Sap with hexosediphosphate. ◑ Sap with ascorbic acid. ● Sap with ascorbic acid and hexosediphosphate.

supply of the coenzyme at a difficult time, we were able to make a trial. A sample of sap (VII) was extracted in the usual way, cleared by barium precipitation and dialysed against glass-distilled water for 42 hr. at 1·2° C., with occasional stirring. No visible changes occurred in the sap and it was then used in a manometric experiment, with the results shown in Fig. 3. The relation between ascorbic acid and hexosediphosphate oxidation is closely similar to that of previous experiments (Figs. 1, 2), but the absolute rates have been very much reduced by the dialysis. Sap VII itself gave an initial rate of ascorbic acid oxidation of 3·5 cu.mm. $O_2$/ml. sap/min. which was reduced after dialysis to 0·17. The all-round reduction of activity is therefore probably attributable at least in part to dissociation of the ascorbic oxidase.

Addition of coenzyme I to this system caused a very large increase in the rate of oxygen uptake. The experiment was continued for 4 hr., by which time the extra $O_2$

uptake attributable to the coenzyme was 41 cu.mm. $O_2$, sufficient to reoxidize 4·8 mg. The amount added was <0·18 mg. It thus appears that the coenzyme was catalysing the oxidation via the ascorbic acid system of substrates present in the sap. On addition of hexosediphosphate, the rate of oxidation was still further increased. Some of the curves in this experiment showed initial irregularities due to faulty mixing (cf. p. 64), and in estimating initial rates of oxidation these have been ignored and the rate deduced from the well-established trends. The effect of this is to underestimate the two faster rates so that the increases shown are minimal:

| | cu.mm. $O_2$/ml. sap/min. |
|---|---|
| Sap + ascorbic acid + coenzyme I + hexosediphosphate | 0·68 |
| Sap + ascorbic acid + coenzyme I | 0·53 |
| Sap + ascorbic acid + hexosediphosphate | 0·40 |
| Sap + ascorbic acid | 0·17 |

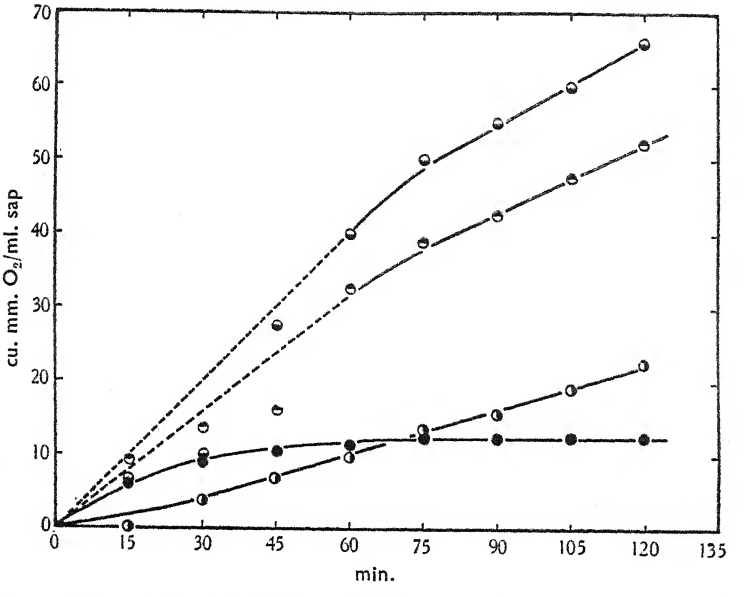



Fig. 3. ◑ Sap with ascorbic acid. ● Sap with ascorbic acid and hexosediphosphate. ◓ Sap with ascorbic acid and coenzyme I. ◒ Sap with ascorbic acid, hexosediphosphate and coenzyme I.

$CO_2$ *emission.* It has been shown earlier that barley saps release $CO_2$ from hexosediphosphate (James & Bunting, 1941); and that no $CO_2$ is formed with ascorbic acid alone (James & Cragg, 1943). The limited number of $CO_2$ determinations carried out in the present series of experiments confirmed these results with the addition that particularly active and incompletely cleared saps might also release $CO_2$ when only ascorbic acid had been added. Table 11 collects these results. In the fully clarified saps addition of ascorbic acid and coenzyme I increased $CO_2$ output from hexosediphosphate. There was also a small $CO_2$ output when coenzyme I was added to ascorbic acid (sap VII). The $CO_2$ output from hexosediphosphate alone was unaccompanied by $O_2$ absorption in the Ba-cleared sap (II) and in the dialysed sap (VII) $CO_2$ output exceeded $O_2$ consumption, when hexosediphosphate was present. In all other cases the $CO_2$ evolved was less than the $O_2$ taken up. The $CO_2$ data are complex and obviously inadequate for a detailed analysis, but the increased output confirms the oxidation of hexose-

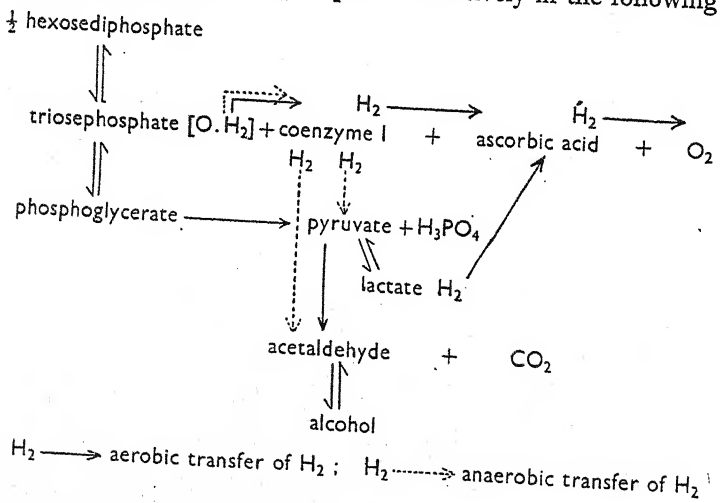
diphosphate via coenzyme I and ascorbic acid. $CO_2$ formation from hexosediphosphate in the absence of added catalysts and with no $O_2$ absorption suggests that there is an anaerobic breakdown also. The results further indicate that the oxidation catalysed by the ascorbic acid system is not directly concerned with $CO_2$ formation, since the latter has no constant relation to $O_2$ absorption.

Table 11. *Total $CO_2$ emitted in cu.mm./ml. sap*

| Sap | With cozymase, hexose-diphosphate and ascorbic acid | With hexose-diphosphate and ascorbic acid | With coenzyme I and ascorbic acid | With hexose-diphosphate | With ascorbic acid |
|---|---|---|---|---|---|
| Barium ppt. removed: | | | | | |
| II | — | 87·1 | — | 65·0 | 83·6 |
| V | 66·1 | 44·7 | — | — | 0 |
| VII | 117·8 | 98·6 | 10·5 | — | 0 |
| Centrifuged only: | | | | | |
| II | — | 70·5 | — | 14·8 | 0 |
| IV | — | 58·0 | — | 73·0 | 23·0 |

## DISCUSSION

A review of the foregoing data and those from previous papers leads to the suggestion that barley may decompose hexosediphosphate oxidatively in the following reactions:




The evidence at present available for the existence of each of the components may be briefly summarized.

*Hexosediphosphate⇌triosephosphate.* Addition of hexosediphosphate to barley saps leads to the formation of esters which are very unstable in the presence of alkali. This formation is encouraged by addition of small quantities of iodoacetate, a specific inhibitor of triosephosphate oxidation; and even by $M/55$ NaF, which inhibits at the next stage. Greater accumulation is caused by addition of $M/100$ HCN and $N/10$ $NaHSO_3$ which form inactive compounds with the carbonyl of the triosephosphate groups (p. 67). The difference between the results obtained with enzyme inhibitors and the fixatives is likely to be due to the reversibility of the hexosediphosphate⇌triosephosphate reaction.

*Triosephosphate⇌phosphoglycerate.* When saps are incubated with hexosediphosphate and poisons tending to arrest its decomposition at the triosephosphate stage, there is no accumulation of 'unhydrolysable' ($P_u$) phosphate (i.e. unhydrolysable by $N$ $H_2SO_4$ in 3 hr. at 100° C.) or of its ratio to the amount of phosphate hydrolysable in 3 hr. ($P_{180}$). In similar digests poisoned with NaF, the $P_u$ value may rise markedly and the $P_u/P_{180}$ ratio does so consistently. These results may be in part due to the formation of hexose-6-phosphates which are not hydrolysed under the standard conditions. There is, however, no simple relation between the amount of unhydrolysable and inorganic phosphate formed, such as the partial hydrolysis of hexosediphosphate to monophosphate would demand. Moreover, NaF does not inhibit this hydrolysis at the concentrations used. The only substance known whose formation would satisfy these requirements is phosphoglycerate. It was further found that, after incubation but not before, a large proportion of the phosphate that could be precipitated by Ba at *p*H 3 was esterified. This would also point to its existence as phosphoglycerate.

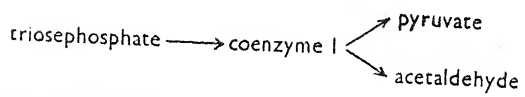
*The ascorbic acid system. Coenzyme I.* The evidence for the existence of an active ascorbic oxidase in barley saps has been detailed in a previous paper (James & Cragg, 1943). It was further shown that the system vigorously oxidized lactic and other α-hydroxy acids without addition of coenzyme. It did not oxidize alcohol. It is uncertain whether our lactic dehydrogenase involves coenzyme I or not; the lactic dehydrogenase of animal tissues does; but that of yeast is said not to (Green & Brosteaux, 1936) and for the present we have to leave open the possibility of an enzyme working with or without coenzyme and a corresponding ambiguity in the summary above. The experiment summarized in Fig. 3 shows clearly that $H_2$ transfer may take place between coenzyme I and ascorbic acid in our saps and that substrates in the sap may be actively oxidized by this means.

*Hexosediphosphate and ascorbic acid.* The most significant result of our experiments lies in the fact that they establish a link between a well-investigated oxidation system and an important product of glycolysis. So far as we are aware this has not been established before in any of the higher plants. It is especially of interest because the oxidation system in question is typical of plants and is not known to function in animals. Glycolysis, on the other hand, appears to proceed by similar stages in both.

Evidence of the connexion between glycolysis and oxidation via the ascorbic acid system is afforded by the following data. Addition of ascorbic acid increases loss of hexosediphosphate and gain of the unhydrolysable ester presumed to be phosphoglycerate (p. 65). This takes place in the presence of NaF; but the additional effect of ascorbic acid is inhibited by traces of $Cu^{++}$, which allows triosephosphate to accumulate in our saps (p. 66), but irreversibly oxidizes the ascorbic acid (*p*H 6). A marked increase of $O_2$ uptake was noted in the presence of the Cu. Approaching the system from the other end, we found that addition of hexosediphosphate to clarified saps only caused $O_2$ consumption if ascorbic acid also was added; and then increased the rate well above that caused by the ascorbic acid alone. In fully clarified saps ascorbic acid and coenzyme I increased the $CO_2$ output from hexosediphosphate. As hexosediphosphate is not an easily oxidized substance, we presume that the readily oxidizable triosephosphate produced from it is the substance actually dehydrogenated. If it were some later glycolysis product we should expect the phosphoglycerate fraction to be decreased instead of increased by ascorbic acid; or that its effect would be inhibited by NaF.

We have indicated the accession of the elements of water to triosephosphate in a general way only, as we do not know whether it proceeds by hydration or by phosphorylation, as in yeast, or by some other means.

In the absence of ascorbic acid hexosediphosphate is broken down to pyruvic acid (James, James & Bunting, 1941) which may be decarboxylated to acetaldehyde and $CO_2$ (James & Norval, 1938). These reactions will occur anaerobically and, when ascorbic acid is absent, without $O_2$ uptake in air (p. 72). Both lactate and alcohol are known to be produced by barley if $O_2$ uptake is prevented. It is highly probable that they arise by the reduction by coenzyme I of the pyruvate and acetaldehyde which we have shown to be formed. When $O_2$ is present, the normal course of $H_2$ transfer is triosephosphate → coenzyme I → ascorbic acid → $O_2$; but when $O_2$ or ascorbic acid are removed it necessarily becomes deflected to the internal $H_2$ acceptors:




Neither pyruvate nor acetaldehyde accumulate under aerobic conditions and their fate is still an open question.

## SUMMARY

1. Barley saps incubated for 24 hr. at 30° C. with hexosediphosphate were analysed for phosphate fractions. In the presence of thymol and NaF they showed a loss of hexosediphosphate and a rise in the ratio of unhydrolysable/3 hr.-hydrolysable phosphate. Reasons are given for taking the unhydrolysable ester formed to be phosphoglycerate.

2. This effect was increased by addition of ascorbic acid, but the presence of $M/2200$ $CuSO_4$ abolished its action.

3. In the presence of iodoacetate or NaF the digests accumulated alkali-labile phosphate esters (triosephosphates). Cyanide and bisulphite increased this accumulation and phosphoglycerate was not formed.

4. In manometric experiments, clarified saps with hexosediphosphate absorbed little or no $O_2$. On addition of ascorbic acid, rapid $O_2$ uptake occurred in excess of the oxygen uptake caused by ascorbic acid alone.

5. Addition of coenzyme I to sap with ascorbic acid caused a large increase of $O_2$ uptake, far beyond the $O_2$ equivalent of the coenzyme added. Hexosediphosphate still further increased this $O_2$ uptake.

6. The linkage between the oxidation and glycolysis stages of respiration suggested by these results is elaborated on p. 72.

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# REVIEWS

*Aquatic Phycomycetes (exclusive of the Saprolegniaceae and Pythium)*. By FREDERICK K. SPARROW, Jr. $9\frac{1}{2} \times 6$ in. Pp. xix + 785, with 1 plate and 69 text-figures. University of Michigan Press. 1943. Price $5.00.

Modern mycology, ushered in by the brilliant researches of Anton de Bary, is now just about a century old, and mycologists naturally look back on the past hundred years and reflect on the growth of their science. Such reflexion cannot fail to reveal one outstanding fact—the shifting of the centre of gravity from Continental Europe to the New World. In this century North America has produced more outstanding mycologists than the rest of the world put together. It would be straying too far to attempt to analyse the causes of the American lead, but suffice it to say that in America mycology is a vigorous and growing science. Especially conspicuous is American pre-eminence in the study of aquatic fungi. A list of those who, in recent years, have made major contributions to the study of these organisms is a long one: Weston, Couch, Coker, Karling, Emerson, Kanouse, Matthews, Berdan and, very recently, J. R. Raper, but probably the man who, more than any other, has the widest acquaintance with aquatic Phycomycetes is Dr F. K. Sparrow. For the past fifteen years mycological literature has been enriched by contributions from him at an average rate of more than two a year, and now, to his great credit, he has laboriously compiled a most complete systematic account of these fungi together with exhaustive references to all the relevant literature.

Before passing on to a discussion of certain points of detail, it may be well briefly to describe the plan of the book. There is a 20-page introduction dealing with the occurrence, collection and classification into orders of the aquatic Phycomycetes. This is followed by an account of each of the eight orders into which these fungi are divided. For each order there is a general discussion, which for the Chytridiales covers 52 pages, followed by the purely systematic part. Although 475 species included in 112 genera are considered, each species is given a full description and its known geographical distribution in the world is clearly indicated. The book is richly endowed with artificial keys to the genera of the various families and to the species of each genus. There is even a key to the species of *Rhizophydium*, the most difficult and heart-breaking genus with which the student of chytrids has to contend, but, in fairness to the author, it must be said that he fully emphasizes the uncertainty of specific identifications in this, the largest, genus of the Chytridiales. The figures are excellent, a very large proportion being from the author's own papers.

Some of us, reared on older schemes of classification, may not feel quite at home in the system used by Sparrow. The grouping of orders is not into ARCHIMYCETES (a dust-bin group of which it is good to see the end) and OOMYCETES, but into UNIFLAGELLATAE (containing species with posteriorly uniflagellate zoospores) and BIFLAGELLATAE (with the zoids anteriorly biflagellate). The first division contains the Chytridiales, Monoblepharidales and Blastocladiales; the second the Plasmodiophorales, Saprolegniales, Leptomitales, Lagenidiales and Peronosporales. The scheme, on the whole, seems much more natural than its predecessors, although the proper position of the Plasmodiophorales must still be considered in doubt.

A point which is of fundamental importance and which the author stresses is the parallel evolution in the UNIFLAGELLATAE and the BIFLAGELLATAE. In both there are olpidioid types (*Olpidium* and *Olpidiopsis*); in both monocentric rhizidiaceous types (*Rhizophydium* and *Thraustochytrium*); in both types where the thallus is converted finally into a chain of olpidioid sporangia (*Septolpidium* and *Sirolpidium*); in both arborescent coenocytes of limited growth (*Blastocladia* and *Rhipidium*); and in both coenocytic mycelial types of more or less unlimited growth (*Monoblepharis* and *Saprolegnia*). A few aquatic Phycomycetes do not fit well into the new scheme. *Rhizidiomyces* and *Hyphochytrium*, both with anteriorly uniflagellate zoospores, are treated in the family Hyphochytriaceae which is given as a 'family of uncertain affinities'. Perhaps 'uncertain' is rather an unfortunate word since there is no certainty concerning any affinities in the fungi.

Although the new classification is to be welcomed as introducing a more reasonable arrangement of these fungi, there are certain points of detail which may not commend themselves to all. It seems rather straining the Saprolegniales to include the microscopic monocentric fungi of the Ectrogellaceae, whilst the Leptomitaceae, so obviously near to the Saprolegniaceae, is banished to a separate order.

In the Chytridiales, with which more than half the book is concerned, a primary division is made into two series: OPERCULATAE, in which the sporangium dehisces by a lid, and INOPERCULATAE. One result of this is to break up the old family Rhizidiaceae which is now replaced by three families: Phlyctidiaceae, Rhizidiaceae and Chytridiaceae. Although the case for this new arrangement may be very good, the reviewer regrets the disappearance of the convenient wider conception of the Rhizidiaceae embracing all monocentric chytrids with a rhizoidal system.

Although Dr Sparrow's book is a monographic account intended for the research worker, the undergraduate, reading for an honours degree in botany and specializing in mycology, will find the introduction to each order very useful. Further it is much to be hoped that the system of classification of the Phycomycetes suggested in the book will be introduced for university students in this country, even though this may involve the disappearance of such a time-honoured name as 'Oomycetes'.

A book of such size must take a very long time to write, and, once written, its production, especially in these times of crisis, is a lengthy business. It should be noted that the preface is dated December 1940, but the date of publication is 1943, and during the past two years considerable contributions have been made to the subject. However, references to literature published since 1940 have been inserted, probably during the 'proof' stage of production, as footnotes. The difficulty about a book concerning a rapidly expanding subject is that it is to some extent out of date when it is published, and in five years' time it may be a rather incomplete account. Mycologists must, therefore, hope that Dr Sparrow will from time to time, say every five years, issue supplements to bring his monograph up to date. Such a procedure would probably be more satisfactory and much less costly for everybody, than the issue of new editions.

Much remains to be found out about the microscopic aquatic Phycomycetes and now, with this book, the identification of a given species is relatively simple. This should commend these fungi for study not only to the academic mycologist, but also to the keen amateur with a good microscope and with a capacity for making minute observation.

A worker turning his attention to chytrids will soon discover that a number of his species find no mention in Dr Sparrow's monograph, because probably more than one species in ten which he finds will be new to science. This great likelihood of discovering new species will, doubtless, be an incentive to some workers.

The publication of this fine monograph represents a consolidation of the position in our attack on the mysteries of these beautiful fungi, and now many should be stimulated to advance still further the front of our knowledge.

C. T. INGOLD

*The Permeability of Natural Membranes.* By H. DAVSON and J. F. DANIELLI. $8\frac{1}{2} \times 5\frac{1}{2}$ in. Pp. x+361, with 73 text-figures. Cambridge University Press. 1943. Price 25*s*.

A text-book on permeability may be limited to discussion of the physical chemistry of diffusion through membranes or it may deal with all exchanges of substances between tissues and the environment many of which are closely linked with metabolic processes. Certain phrases in Drs Davson's and Danielli's book suggest that they would prefer to limit discussion to the former strict field; a chapter on secretion and one on the kidney are however included.

A sentence in the preface, 'The last twenty years have seen a steady development of exact measurements of membrane permeability, mainly due to the American Schools of Lillie, Lucke and McCutcheon, and Jacobs, but also contributed to by the Finnish school of Collander and Bärlund', gives the impression that the extensive plant physiological literature has been neglected. The neglect is not so marked as this sentence suggests but there is considerable bias in treatment. The membranes dealt with are the lipoid plasma membranes of the outside of the protoplast. Heterogeneous or porous membranes like plant cell walls are not discussed though there is a rather brief mention of diffusion potentials across artificial heterogeneous membranes.

The early introductory chapters deal largely with the osmotic system of the cell and with methods of permeability measurement chiefly of animal cells. There follows a particularly valuable discussion of diffusion processes through liquids and liquid membranes; the theory of diffusion through lipoid membranes is relegated to an appendix, but the use of quantitative tests of membrane structure based on this general theory are dealt with at some length in chapters V, VII, and XXI and are alone a justification for the book.

The general theory of diffusion through lipoid membranes, though admittedly incomplete, has enabled the authors to collate into a logical system a large body of data on permeability of plant

and animal cells to water, ions, non-electrolytes and large molecules. The permeability data are extended by those of impedance and potential measurements and by information regarding the surface of the cell which general consideration of the physical chemistry of surfaces has afforded. All of this discussion gives a well-balanced picture of recent work on the structure of the external plasma membrane and its permeability relations.

An important part of the picture is Dr Danielli's theory of diffusion through lipoid films which one regrets is rather tersely dealt with. Evaluations of constants defining diffusion of the type

$$a = r\phi_a \frac{\sqrt{(RT)}}{2\pi M} e^{-\mu a/RT}$$

are scarcely explained, which seems unfortunate when rather detailed explanations are given of $P = RT.C$ (the gas laws applied to dilute solutions) in an earlier chapter.

The two chapters on secretion and the kidney (making up 10% of the book) seem to lie rather outside the main argument of the book, but one cannot complain that they are uninteresting. Discussion of hypothetical mechanisms inevitably bulks large in any treatment of fundamental principles underlying secretion; this is the aspect dealt with. Incidentally, Schreinemakers is rather incompletely quoted on p. 280, where it is implied that his work shows that anomalous diffusion produces hypotonic solutions; this represents only one of his diffusion types, hypertonic solutions can also be produced.

There are a few numerical misprints which readers will notice without difficulty, such as, $10^{-5}$ instead of $10^5$ and 0·05 instead of 0·005 on p. 329, etc. Several citation numbers of equations appear to be wrongly given, but the readability is not impaired by these slips.

Authors and publishers are to be congratulated on a book which satisfactorily surveys the last two decades of work on permeability of plasma membranes. Its value is further increased by inclusion of much of the authors' unpublished work and by new treatment of temperature coefficients of diffusion and development of tests of membrane structure. T. A. BENNET-CLARK

*Die Ostgrenze Fennoskandiens in pflanzengeographischer Beziehung.* By AARNO KALELA. 9 × 6 in. Pp. 68, with 4 text-figures. Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich, Heft 20. Bern: Hans Huber, 1943. Price 4.20 Swiss frs.

Since the time of William Ramsay the concept of 'Fennoskandia' as a natural region has been familiar and accepted: where now must its eastern boundary lie? The White Sea forms an evident boundary in the north, where the evidence of geology, physiography, phytogeography and zoogeography coincide to emphasize the discontinuity. Farther south, however, the line of the boundary has been more disputable. Cajander indicated that it happens very closely to follow the present state frontier of Finland, running just west of the Onega river from the White Sea southwards to the southern end of Lake Onega, and thence westwards to the Gulf of Finland.

There is a rather sudden increase in continentality of climate at the state boundary. The rock formations east of it are of great age, and are mostly granitic: from their surface all old soils and weathered rock were swept away by the Ice Age, which, however, itself left a thick cover of glacial material which now constitutes the intricate background to a multitude of scattered lakes and twisting waterways. In contrast, the west Russian lands are of horizontal sedimentary rocks, relatively little covered by glacial material and with few lakes and streams. The Finnish soils are generally acidic and base-deficient; the Russian calcareous. This boundary also represents a sudden diminution westwards of the easterly Russian floral element, and a corresponding diminution eastwards of the westerly element. The Siberian larch, *Larix sibirica* Led., is closely restricted by the same boundary, which also more roughly serves *Abies sibirica* and *Pinus cembra* spp. *sibirica*. Kalela gives lists of shrubs and herbs which have a similar westward restriction, and their total of 133 flowering plants seriously affected in distribution at the Finnish border is a substantial figure beside the low total for the whole of the Finnish flora. Of westerly floral elements thinning out east of the boundary Kalela is able to cite some from the conifer zone and more from the northern tundra and fjell.

The long low watershed running south through the middle of Finland from Petsamo, he demonstrates to be a boundary also between eastern and western floral elements, but one of far less importance.

Though the climatic changes from west to east are large, they are fairly gradual and will not explain the sharpness of the floristic boundary. The great and sudden geological differences at this line may be taken as emphasizing very greatly the floristic differences on the two sides of

it, the habitat ranges available to east and west being very different, especially in respect of lime content of the soil. This is readily understandable when we recall that towards the limits of their climatic range species become more sensitive to the edaphic factors of their environment.

It is now recognized that north Russia and the Kanin peninsula were ice-free during the glacial period, and Nordhagen has shown good grounds for supposing that there was an ice-free fringe to the Norwegian coast. Here doubtless the tundra and arctic fjell plants survived. Apart from this and very recently evolved species the whole Fennoskandian flora must have immigrated in the post-glacial period. The chief routes of immigration from the east were across the mouth of the White Sea (formerly less wide), and up the Aunus and Äänis necks of land, but everywhere the invading plant host must have been severely sorted out at the boundary. From the west tundra, fjell, and wood floras must have migrated, since early post-glacial times, by the Norwegian coastal strip and the Kola peninsula. Not until much later were the Baltic shores open for migration from the west.

It is shown that no less than the species themselves, the plant communities of east Finland and west Russia differ much from one another, a circumstance which whilst clearly to be expected from the edaphic and climatic dispositions already described, does nevertheless add force to the author's localization of the east Fennoskandian boundary.

Convincing as this brief exposition is, one cannot help regretting that such evidence as pollen analysis and peat investigations afford has not been collected and added to the account: after all, in this way alone have we the clinching record of the actual former dispositions of any flora.

H. GODWIN

*A Hand-book of Plant-Tissues Culture.* By P. R. WHITE. $8\frac{1}{4}\times5\frac{1}{2}$ in. Pp. 277, with 71 text-figures and plates. Lancaster, Pennsylvania: Jaques Cattell Press. Price $3·75.

The difference between the organization of the higher plant and that of the animal is indeed clearly emphasized in this latest addition to the small but growing library of books on tissue culture. To one accustomed to the behaviour of animal tissues, and justly criticized by the author for his ignorance of even the rudiments of plant-tissue culture, this volume comes as a welcome addition. It explains clearly what can and what cannot be achieved with plant tissues in culture, and, moreover, gives precise instructions for anyone to follow who may wish to put the subject into practice. These instructions are both clear for the beginner, unaccustomed to any form of culture technique, and also adequate for the lesser tyro.

Perhaps one of the most outstanding differences between the plant and the animal culture brought into prominence by the techniques so far developed is the macroscopic character of the former as opposed to the microscopic character of the latter. This may only be a phase which will disappear with the further elaboration of the two types of technique, but at the same time it has largely determined the fields in which results have so far been achieved. The fact that it is possible to cultivate plant tissues up to quite large dimensions without the necessity to subculture at frequent intervals has allowed much more progress to be made in the provision of suitable nutrient media, and the relative simplicity of the constituents of these solutions has no doubt contributed to the achievement of that satisfactory state in which it is possible to maintain cultures alive on media which can be completely described in terms of pure chemicals. This being so, and considering also that meristematic tissue is the only type which can be successfully investigated, it is surprising to find that so little has yet been achieved in plant-tissue culture in the direction of elucidating the determining factors in the onset of cell division. The measurement of growth by means of a foot-rule strikes one accustomed to the measurement of growth by cell counts or by the nucleoprotein phosphorus content of the tissues as a somewhat rough-and-ready procedure. However, both methods seem to yield results, and only the future will assess them at their true values. There seems to be still a great field for research by the tissue-culture method into the action of plant hormones under more controlled conditions than can be obtained in the whole plant, and for studying the relationship between the hormones and the gradient systems in the plant. The few data given on the effects of plant hormones on tissue cultures are indeed interesting. Many other promising fields for future research are also indicated and discussed.

The book is well illustrated to the extent of including among the numerous purely botanical figures, portraits of the pioneers in the field of animal-tissue culture. It contains an extensive bibliography which, however, unfortunately seems to obtrude itself in the reading of the text, where in spite of the author's admiration for William of Occam, numbers seem to have been multiplied beyond necessity.

E. N. WILLMER

*An Introduction to Plant Physiology*. By W. O. JAMES. $7\frac{1}{4} \times 4\frac{3}{4}$ in. Pp. viii + 269, with one photograph and 74 text-figures. Oxford University Press. 1943. Fourth Edition. Price 8*s*. 6*d*.

That a fourth edition of Dr James's well-known book has now been published, only twelve years after the first edition made its welcome appearance, is a sufficient recommendation that his method of approaching the subject of plant physiology has been very widely acceptable to students 'of senior school or junior university status', and to their teachers. This reviewer will continue to urge those of his own students who are reading for the intermediate examinations or for the first M.B. to study assiduously the subject-matter of this book, and will continue to bring it to the notice of schools where it is not already used. In every respect it is a worthy successor of the earlier editions. The author and the Clarendon Press deserve our thanks.

About the new matter in the fourth edition the author writes in his preface: 'The section on enzymes and the chapter on respiration have been entirely rewritten, and the bibliography has been brought up to date. Greater emphasis has been put upon the linkage of water loss and gain resulting from the transpiration pull upon absorption.' The distinctive feature of Dr James's treatment of his subject is that he often offers to elementary students fare which, but a few years ago, was reserved for honours students in universities. Especially is this so in the sections on plant chemistry and metabolism. For example, examiners in the Higher Certificate need not be surprised if, from now on, some of the candidates not only show an acquaintance with compounds such as fructo-furanose-1:6-diphosphate, but also express views about 'the Pasteur effect', and other matters which ten years ago were being discussed in the meetings of learned societies.

In a sense Dr James is making an important experiment in the elementary teaching of his subject. The question is this: Are students at this stage sufficiently mature to appreciate and assimilate all the author's statements of fact and the arguments that he develops therefrom? Presumably Dr James thinks that they are; the reviewer hopes that he is right, and will look for evidence during the next few years. What is clear is that the more advanced university students will derive much profit from reading many of the sections, especially those on photosynthesis, nitrogen metabolism and respiration, in which the treatment is coloured by the results of researches that have been carried out by the author or in the author's department. The eighty-six citations in the bibliography will be most valuable to such students and to school teachers. In citation 36, should not the author's name read Green, D. E.?

The seventy-one experiments described in the first edition, which were rightly commended by a reviewer in the *New Phytologist*, **31**, 1932, 71, remain unchanged. It is rather surprising that a few simple experiments illustrating the activities of some of the different oxidation enzymes have not been added. Further, there is no experiment proving that oxygen is absorbed in aerobic respiration, although the results of Exp. 28 are interpreted on the assumption that oxygen has been absorbed. Finally, one question about the old text must be put. Does the author wish the reader to infer from the statements on pp. 131–2 and 158 that a sucrose solution is colloid? Exp. 52 on plasmolysis shows that sucrose molecules penetrate a cell wall; and in the reviewer's experience they pass, albeit slowly, through a parchment membrane. For the elementary student the point is obviously important in deciding whether sucrose is to be regarded as one of the sugars which 'are able to move about inside the fabric of complex plants' (p. 177). M. THOMAS

*Ecological Crop Geography*. By KARL H. W. KLAGES. $6\frac{1}{2} \times 8\frac{1}{2}$ in. Pp. xviii + 615, with 108 text-figures. New York: The Macmillan Company. 1942. Price $4.50.

This book is concerned with the distribution, yield, and growth of the principal crop plants of the U.S.A., particularly as they are found in that country, but also as they are grown in other parts of the world. The big range of climate, physiography, soil, and racial inheritance in the U.S.A. ensures that this list is a long and comprehensive one.

Broadly speaking, we may say that the author's objective has been to interpret the areal distribution of each crop, not only in terms of economic and social factors, but also in terms of the full ecological background of the crop plant's requirements of, tolerance of, and responses to, the climatic and edaphic conditions. This aim has evidently been followed in the series of university lectures which has been the origin of the book, and which has determined the form and level of presentation.

The first part of the book is termed 'The Social Environment' of crop plants, and in this an

outline is given of the economic, political, social, technological, and historic causes which have in part shaped the distribution of crops throughout the world. Although only 72 pages go to this important field, they suffice to make the student aware of the general character of a big range of operative factors, and to show him an entry into their copious literature. In Part II, 'The Physiological Environment' (58 pages), the author treats of the general aspects of the interactions between plant and environment, discussing, among other topics, growth curves, optimal and critical factors, the concept of 'ecological optimum', the variability of crop yields, etc., particular stress being placed on the demonstration that low variability in yield from season to season is a factor strongly favouring success of a crop, and one notably lacking in the marginal climatic areas. Part III, which is much longer, is called 'Ecological Factors', and deals with the soil and atmospheric humidity relations of plants, and temperature and light relationships. In each of these vast fields discussion begins with the basic physiological processes affected by the environmental factors, and whilst the treatment here is not very precise or critical, the exposition is sufficiently informed. Upon this basis the author proceeds to consider various schemes in use for classifying and mapping areas in relation to the manifold environmental factors. Thus in relation to humidity he refers to direct precipitation and evaporation data, to Meyer's $N$-$S$ ratio, to Lang's rain factor, to de Martonne's index of aridity, and to Thornthwaite's precipitation effectiveness index. He finally proceeds to the classification of climate, explaining Köppen's and Thornthwaite's systems in particular reference to the U.S.A. Throughout this chapter Prof. Klages illustrates his argument by constant reference to the behaviour of crop plants under the influence of given factors.

The last and largest section of the book is Part IV (260 pages), in which all the crop plants are considered one by one: in a sense all three preceding parts are merely preparation for the synthesis of this part. The particular value of the accounts in this part lies in the fact that not only do they give carefully prepared statements and maps of the distribution of each crop, but an informed comment on the factors operating to determine it, and a comment at that which takes in fully the ecological adjustment of the crop plants. These accounts are based on the experience of practising agriculturists as well as the experiment and observation of agricultural scientists. The result in many instances is a collection of valuable ecological information, such as we possess at present for no wild plants. This is especially so for the climatic response of related varieties of plants. Although the author does not himself set out to show this, a very interesting contrast emerges between the operation of ecological factors determining the distribution of a wild plant, and the operation of similar factors acting in a more complex way together with human factors, to determine the distribution of a cultivated plant.

Prof. Klages's book will be welcome to botanists not only for its wealth of ecological information, but also for the promise it gives of applying ecological methods increasingly to the study of crops, and for expansion of the new field of study between ecology and economic botany. A very attractive feature of the book is the constant reference to fundamental German scientific work, and the frequency with which the German literature is cited in the bibliographies. H. GODWIN

# THE ROOTS OF *EQUISETUM LIMOSUM* L.

BY JOHN WALTON, M.A., D.Sc.
*University of Glasgow*

(With 5 figures in the text)

The striking formality of the architecture of the shoot of *Equisetum* and its close dependence on the regular segmentation of the three-sided apical cell have attracted the attention of many morphologists, and from the early years of the nineteenth century a succession of investigators have demonstrated many interesting facts about the structure and development of this remarkable type of plant. Less attention has been given to the root system and roots.

Janczewski (1876) demonstrated that the lateral buds, which produce the branches of the rhizome and aerial shoots, and the roots, arise superficially at the bases of the internodes but are covered by the leaf sheath belonging to the internode below. The buds alternate in position with the constituent leaves of that leaf sheath. The branches and roots produced by these buds perforate the base of the leaf sheath surrounding them and appear to emerge endogenously from its base. Some of the buds, particularly those on the aerial parts of the shoots, produce under normal circumstances branches and no roots, but if the shoot is allowed to develop in a damp atmosphere roots may be developed instead. On the underground parts of the shoot system the bud may develop a branch apex, and from the base of the bud one or more roots may develop. Sometimes this bud forms a rhizome or an erect aerial shoot or its apex may abort and the bud produce roots only. Duval-Jouve (1864, p. 5) has noted that, while most *Equisetum* roots are less than 1 mm. in diameter, in *E. ramosissimum* and *E. hiemale* some positively geotropic roots occur which are over 2 m. in length and up to 4 mm. in diameter. Russow (1872, p. 147) mentions roots of *E. limosum* 2–2·5 mm. in diameter which have a five-rayed core of xylem containing spiral and annular elements. In 1928 the author gave a preliminary account of the structure of the large roots of *E. limosum* and also recorded the presence of similar large roots in the species *E. maximum*, *E. sylvaticum* and *E. arvense*.

Most of the roots of *Equisetum* have a single large central tracheid with two or three protoxylems each represented by a single tracheid, equidistantly arranged around it. In this respect it differs greatly from the roots of the extinct Equisetales which have from two to twenty-five or more protoxylems, and a fuller description of the larger *Equisetum* roots would appear to be justified as they tend to resemble those of the Calamitean type very closely. In addition, the anatomy of the vascular connexion between the large type of *Equisetum* root and the shoot that bears it shows features of special interest.

## STRUCTURE OF THE ROOTS OF *EQUISETUM LIMOSUM* L.

This investigation was based on plants of *E. limosum* found on a deeply silted area by the river in Borrowdale, Cumberland. Large portions of rhizomes with their roots were dug up. The main rhizomes (diam. 11 mm.) were horizontal and about 30 cm. below ground level in waterlogged silt which made the removal of the large deep-growing roots a difficult task, and as a result no root tips of the largest size of roots were obtained.

These large roots extended vertically down to a depth of over a metre from the surface. A piece of rhizome bearing one lateral shoot and numerous roots is shown in Fig. 1. It was noticed that the small roots were ageotropic while the larger roots (2–5 mm. in diam.) were positively geotropic. These larger roots are only found on the horizontal parts of the rhizomes.

The structure of the small roots of *Equisetum* is sufficiently well known (van Tieghem, 1870, p. 76) to require but a brief review. In the mature part of the root the surface is covered by the piliferous layer with rather sparse root hairs, the outer three or four layers of the cortex are usually heavily lignified while the inner cortex consists of parenchyma with intercellular spaces. In some roots these spaces may be quite large. The endodermis which has a well-developed Casparian strip has arisen by periclinal division of a layer which has given rise to the endodermis and an inner layer corresponding to a pericycle.

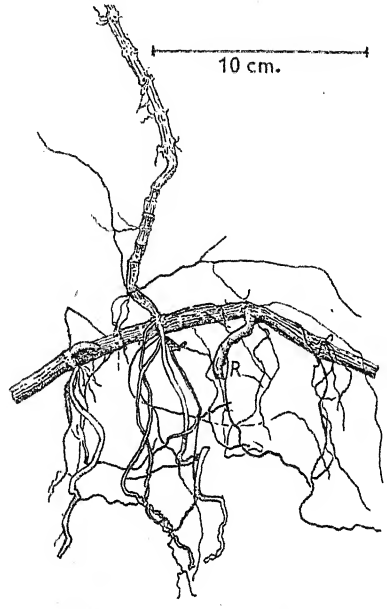


Fig. 1. Part of a rhizome of *E. limosum* with base of an aerial shoot and roots of various sizes. At *R* is part of a root 4 mm. diam.

From this inner layer the lateral roots originate. There are also small intercellular spaces between the endodermis and the pericycle at the cell corners. Inside the pericycle are the three protoxylem elements alternating with strands of sieve tubes. The protoxylems alternate with the cells of the pericyclic layer and there appear to be no passage cells in the endodermis. In the centre of the root is a single large metaxylem element with which the three protoxylems are in contact.

The large roots differ from the small in the greater development of lacunae in the inner cortex and in the greater development of the stele. In the largest roots investigated (Fig. 2 A) the inner cortex is split in a schizogenous manner into vertical radial plates of parenchyma, and this results in a highly developed ventilating system. In roots with a diameter of about 1 mm. there is usually an unlignified cell between each protoxylem element and the central metaxylem tracheid. This separation of the protoxylem from the metaxylem is paralleled in the stem where the metaxylem in the internodal bundle is

usually separated from the carinal canal by some parenchymatous elements. In roots with a diameter of about 1·3 mm. there are usually additional metaxylem elements between the protoxylems and the central tracheid which may not differ from them in size. Roots with a diameter of 2·5 mm. are usually tetrarch and have about ten metaxylem elements.

The largest roots sectioned were about 4 mm. in diameter and have a hexarch stele (Fig. 2 A, B) containing about sixteen metaxylem elements. Measurement of roots of various sizes from *E. limosum* show that the total number of tracheids in the stele is directly proportional to the diameter of the root.

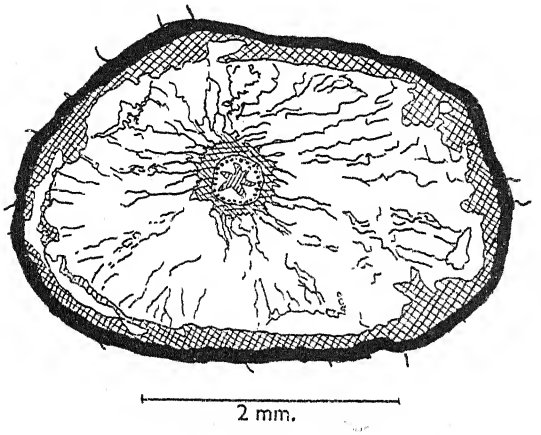


Fig. 2A.

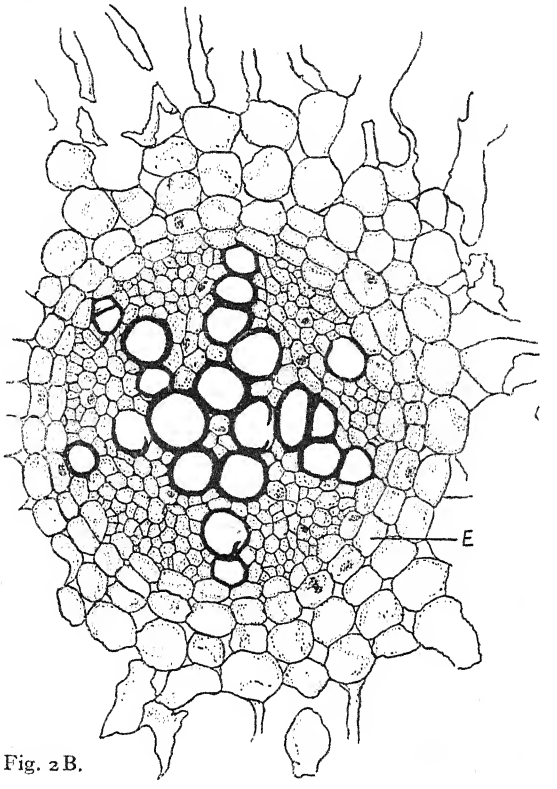


Fig. 2B.

Fig. 2. A. Diagrammatic representation of cross-section of a large root of *E. limosum*. Sclerenchyma black, parenchyma crosshatched, endodermis dotted line, xylem in the centre. B. Stele and adjacent cortical tissues of the root shown in A from a camera lucida drawing × 120. *E*, endodermis.

### The vascular connexion between large root and rhizome

At the node of the rhizome (Fig. 3) the vascular connexions between the internodal strands and the leaf traces are as described by Gwynne-Vaughan (1901) and Barrett (1920, p. 221) for *E. arvense* and *E. maximum*. The protoxylem of the internodal strand is linked with the vascular strand of the leaf above. There appears to be no connexion between the protoxylems of one internode and those of the internodes above or below. The metaxylem, on the other hand, poorly developed in the internodal bundle, increases in amount at the node and becomes continuous with that of adjacent strands so that a continuous ring of metaxylem is found at the node. The vascular supply to each lateral bud or root is

attached to this nodal metaxylem half-way between a pair of leaf traces so that the roots and buds alternate in position with the leaves at the node. Where the bud aborts or where only a small root is attached the form of the nodal ring of metaxylem is not affected by the attachment of its vascular supply, but where there is a large root with a bud there is a considerable modification of the nodal ring in relation to the vascular supply to the lateral organs as may be seen by comparing Fig. 3 with the diagrams given by Gwynne-Vaughan (see Bower, 1935, fig. 134) and Barrett (1920, text-fig. 8). In Fig. 4 is a diagrammatic representation of a longitudinal radial section through the node of which a recon-

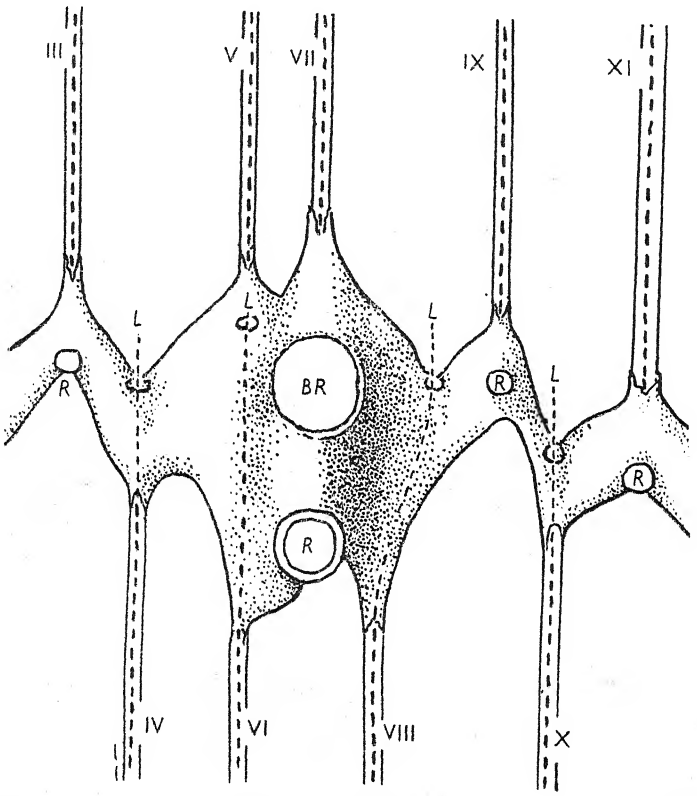


Fig. 3. Diagrammatic representation of part of the xylem system of a node of *E. limosum*. Roman numerals indicate internodal vascular bundles; even numbers those of one internode, uneven numbers those of the internode above. *BR*, insertion of branch bud; *LL*, leaf traces; *RR*, insertion of roots.

struction of the nodal system is given in Fig. 3 showing the attachment of the vascular supply to the root.

The peculiar feature of this node is the form of the vascular connexion with the root. Just below the bud the stele of the root and the part linking the root, bud and rhizome is tubular and is in fact solenostelic with an inner as well as an outer endodermis. There are no spiral protoxylem elements present in the xylem. The sclerenchyma which surrounds the inner sides of the nodal vascular system of the rhizome is continuous with sclerenchyma which projects down into the bud and base of the large root. A cross-section (Fig. 5) of the proximal 2 mm. of the base of the root shows a central strand of sclerenchyma in the centre of a solenostele which has an inner and outer endodermis.

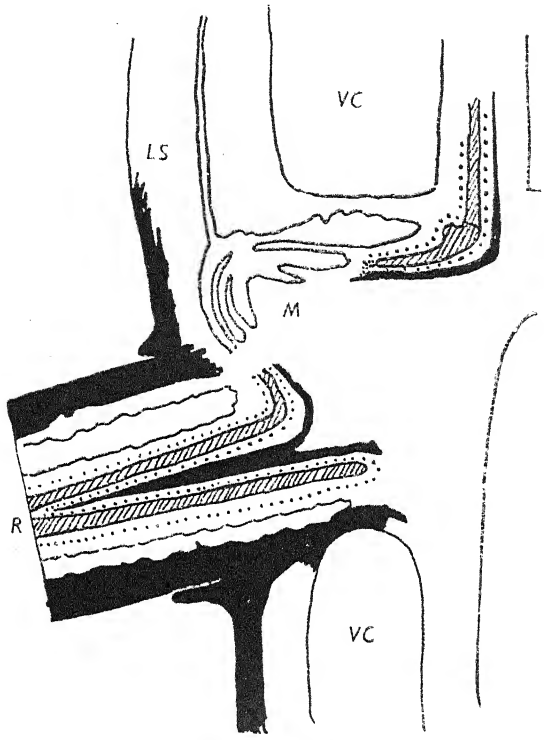


Fig. 4. *E. limosum.* Diagrammatic. Longitudinal radial section through lateral bud and node of rhizome. *M*, aborted branch apex; *LS*, leaf sheath; *VC*, vallecular canals; *R*, root.

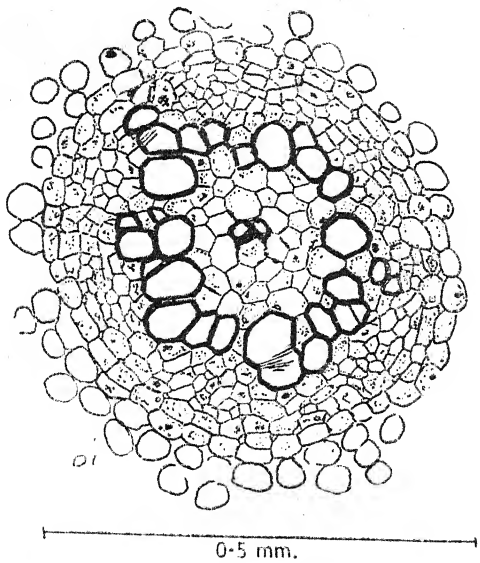


Fig. 5. *E. limosum.* Transverse section through root near *R* in Fig. 4. Showing the stele and part of the adjacent cortex. There are two sclerenchymatous cells in the centre immediately surrounded by an endodermis. The other tissues as in Fig. 2 B.

Distally this sclerenchyma thins out and about 2 mm. from its base the root has a normal root structure. The solenostelic region is evidently of the nature of a transition region between the bud axis and the root.

In the bud axis there is a tubular condition of the vascular system somewhat similar to that found by Barrett (1920, figs. 1 and 7) in the primary and secondary axes of the sporeling plant of *E. arvense*. This supports the suggestion made by Barrett that the internodal system of separate bundles found in the internodes of *Equisetum* has been derived from a protostele through the intermediate stages of siphonostele and solenostele. The lateral bud in its development is subject to conditions not altogether unlike those to which the embryo is subject in its growth. It is closely invested by the tissues of the main axis and leaf sheath and like the embryo in its early stages has a shoot and root apex and a region through which it derives its sustenance.

## COMPARISON BETWEEN THE ROOTS OF *CALAMITES* AND *EQUISETUM*

Williamson & Scott (1895, p. 683), in their account of the roots of *Calamites*, describe under the name of *Astromyelon* roots ranging from 0·5 to 25 mm. in diameter. Unlike *Equisetum*, those with a diameter of less than a millimetre have diarch xylem and those with a diameter of 0·7 mm. and over may have secondary wood. The roots with diameters between 2 and 5 mm. compare most closely with those of corresponding sizes in *Equisetum*. The superficial layer, the lacunar cortex, the endodermis and pericycle and their relations to one another are similar to the same tissues in *Equisetum*. The xylem of the Calamite root has a larger number of elements and may have secondary xylem supplementing it.

A tetrarch *Astromyelon* (Williamson & Scott, 1895, pl. 15, fig. 5) with a diameter of 2 mm. shows a remarkable resemblance to the tetrarch roots of *Equisetum* which have a diameter of 2·5 mm.

These resemblances emphasize the support which is given to the view that *Calamites* and *Equisetum* are closely related types. The differences in the roots are not greater than those which one would expect to find within a natural order of Angiosperms in which some members were herbaceous and others arborescent. The similarity between tissues of the roots of the extinct and living Equisetales suggests that the environmental conditions under which the roots grew were similar. A suggestion which is supported by geological evidence.

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# DEVELOPMENTAL MORPHOLOGY OF VASCULAR PLANTS

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CONTENTS



## Introduction

It is now almost a century since Hofmeister began the series of developmental studies that laid the foundation of modern plant morphology. Since that time, developmental morphology has passed through periods of popularity, alternating with times of partial eclipse, but has never been entirely neglected. Many improvements in methods and technique have of late years given the subject a new impulse, and considerable progress has been made, both on the purely morphological side and in the application of newer physical, chemical and experimental methods to developmental problems. In the latter field there is now a possibility, by use of the increasing supply of instruments and methods that other sciences continually furnish, of increasing, step by step, our insight into the proximate causes behind specific plant forms and structures. There is perhaps no branch of botanical science that will not benefit materially from such discoveries, so it is not surprising that increasing numbers are turning their attention to this type of research.

For such investigators, a survey of the developmental work so far completed is a necessity, as indeed it is for all botanists who would view the modern work in proper perspective. Such an historical survey, in so far as the growth of the vascular sporophyte is concerned, will be attempted in what follows. Portions of the field have already been admirably covered in a number of reviews, which have been of great service in the present work. The mass of discovery has been so great that there has been difficulty in condensing it within reasonable bounds, and the extensive fields of cytology and early embryology have necessarily been omitted.

## BEGINNINGS OF DEVELOPMENTAL MORPHOLOGY

The latter half of the nineteenth century was a period of great significance in botanical history. Schleiden's *Grundzüge der wissenschaftlichen Botanik* (1842), with its insistence on the use of inductive methods and the necessity for developmental studies, captured the imagination of the younger botanists of the time, and had a profound effect on the course of science. Wolff (1759) had discovered the growing point in plants more than eighty years earlier, and numbers of isolated stages in growth and development had been seen, but it required Schleiden's suggestive challenge, acting on the fertile minds of Hofmeister and his followers, to usher in the fruitful period of developmental morphology that followed.

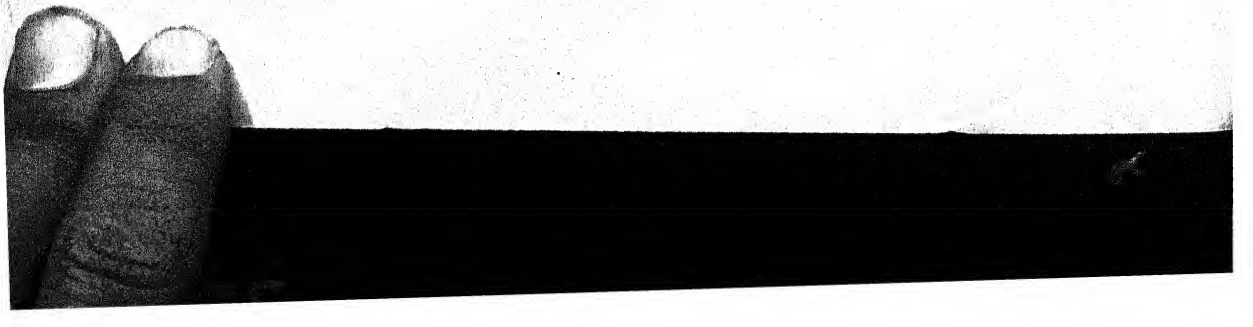
The investigations of this period were not for the most part experimental. They consisted in the accurate observation of successive stages in development under natural conditions and the building of these into a connected ontogenetic history. The results, now matters of text-book information, must have been startling to contemporary botanists. Not only was the true nature of various tissues and organs ascertained to an extent far beyond what had previously been possible, but comparative morphology, based on a firmer foundation, became a real instrument for the study of phylogenetic relationships.

It is perhaps as well that the causal aspect, though by no means absent from the minds of the pioneers, was for the time relegated to a secondary position, for botanical literature was thus relieved of an accumulation of misconception with which it must inevitably have been encumbered had a general attack been directed at causal problems with the insufficient knowledge then available. Instead of that, a broad background of descriptive developmental morphology has been provided, a mass of material invaluable to the student of comparative morphology, and practically indispensable to one who hopes seriously to undertake experimental solution of problems of development.

It was Hofmeister's monumental work, particularly his work on reproduction (1849, 1851, 1862), that demonstrated for all time the value of developmental studies. Before that time many facts concerning the reproductive structures in Archegoniatae and in the seed plants had been discovered. A proper understanding of their meaning was, however, entirely lacking, and morphological thought was in a state of confusion arising from conscientious attempts of the early workers to fit their misconceptions into the natural scheme. For the literature of this aspect of botany prior to Hofmeister's work, Goebel (1926) may be consulted. The situation up to his time may be summarized as follows.

In Bryophyta the archegonium with its neck canal and egg was known, as were also the antheridium and its swimming sperms, but while the view was held in some quarters that antheridia and archegonia were sex organs, there was no suggestion that the egg was fertilized by the sperm, or that it grew into the sporophyte. In fact Valentine, who first discovered the egg, was careful to indicate his conviction that the 'theca' (sporophyte) was completely independent of it, and, while comparing the spores with pollen grains, he maintained that any sexual processes should be sought in the sporophyte capsule.

In ferns, Malpighi had described the sporangia and their method of opening. In 1824 the prothallium was discovered but was thought to be a cotyledon, and even the discovery of antheridia and sperms by Nägeli accomplished little in the direction of immediate understanding, since he failed to see how they could be effective in a sex process which



he thought of as affecting only the spores. The identification of fern archegonia in 1848 merely added another fact to be explained.

In so far as seed plants were concerned, the botanists of the time considered themselves on firmer ground. As long ago as 1695 Camerarius had shown experimentally that pollen was necessary for the production of seed, corroborating the opinions held long before by various observers of reproduction in dioecious plants. A century and a quarter later Amici discovered the pollen tube in *Portulacca*, a discovery almost immediately extended to numerous other plants by various workers. Thus a part of the fertilizing mechanism was made clear, and botanists became divided as to what took place subsequently. One view, vigorously upheld by Schleiden and numerous others, visualized the embryo plant carried into the ovary by the pollen tube and continuing its growth under the favourable conditions provided there. From this arose such diverse explanations of the phenomena observed in lower plants as Leszezyc-Suminski's hypothesis of the growth of fern embryos from the tails of sperms in the archegonia, and Valentine's belief in the homology of the moss protonema with an angiosperm pollen tube, and the complete moss plant with the embryo and seedling into which the pollen tube supposedly developed. The second theory, supported by Amici, Von Mohl, and Hofmeister himself (1847), held that the embryo grew from a cell of the embryo-sac, which became fertilized in some way by the pollen tube.

What was needed was that someone should watch carefully the course of development and record the facts, and that is what Hofmeister did. Tracing ovule development in nineteen angiosperm families (Hofmeister, 1849) he demonstrated the production of egg apparatus and antipodals before the pollen tube had reached the region, and the rise of the embryo from the egg after fertilization. Thus mistaken ideas as to the growth of embryos from pollen tubes were eliminated and a way was opened for the proper understanding of the sexual processes. The development of ovule, embryo, and endosperm was also studied.

Similar developmental studies on mosses, ferns, and gymnosperms (Hofmeister, 1851, 1862) demonstrated alternation of generations, and established the essential similarity of life history on which our concepts of the relationship of these groups are based. In liverworts the whole life history was carefully worked out, with archegonia and antheridia, eggs and sperms, the development of the embryo from the fertilized egg, and the production of spores which grow into new gametophytes. The same was done for the mosses, with more emphasis on development of the more complex sporophyte. Fusion of sperm and egg was not seen, but a sperm was discovered travelling down the neck of the archegonium. Ferns were also found to have two generations, the growth of spore into prothallium, and of fertilized egg into sporophyte being demonstrated. In them he was able to demonstrate the penetration of sperms to the egg (Hofmeister, 1854). Equisetales were shown to behave similarly though the picture was less complete, since gametophytic material was not at the time available. In heterosporous pteridophytes also the alternation of generations was demonstrated, and in gymnosperms the 'endosperm' was recognized as a prothallium, the 'corpuscula' of R. Brown (1844) as archegonia, and the pollen grains as male gametophytes.

The import of this work was so striking, showing clearly for the first time the fundamental unity underlying the diversities of these groups, that developmental morphology was firmly established. With the great advance in methods that has naturally followed,

a host of new discoveries have come to light, all of them serving to fill in and complete the picture outlined by Hofmeister, and there has arisen a complete and self-contained division of morphology confined to gametophytic and embryological development.

This work constituted by no means the whole of Hofmeister's developmental studies. It is a pleasure to read his accurate descriptions of the minute episodes that follow one another in all stages of the plant's development, and there is a constantly increasing wonder at the succession of precise observations he was able to make in spite of the limits imposed by primitive methods. The scrupulous care in observation and the precise record, unmarred by the inadvertent bias that sometimes springs from preconceived hypothesis, render his writings valuable reading for the modern student.

## DEVELOPMENT AT GROWING POINTS

The next stage in the history of developmental morphology, exclusive of embryology, is marked by an interest in growing points, which became intense in the later decades of the last century, and persists to the present time.

### *Apical meristems*

After Nägeli (1845*a*, *b*) had discovered the single apical cell in various algae and mosses, the imagination of botanists was captured by the idea of such single apical cells, dividing according to fixed rule to give rise to the organs and tissues of all plants. Investigations proved that in some cases this hypothesis clearly held, while in others more complexity was encountered. It followed that with a number of botanists loath to abandon the idea that all the higher plants, when properly understood, would be found to conform to Nägeli's scheme, and others in total disagreement with this thesis, the conditions at the growing points of many plants were rather thoroughly investigated. The mass of literature is great, and in cases where the true condition has long been established, individual consideration will be given only to those contributions that are essential to the account.

In the Equisetineae and the greater part of the Filicineae, the single apical cell is agreed by all to be the rule in both stem and root. It is a large cell, pyramidal in shape, and is most commonly triangular in cross-section, though in certain forms it has been shown to have only two sides, curved so the section is lenticular (Poirault, 1890; Zawidzki, 1912; Chang, 1927). In the case of stems, anticlinal divisions cut off tabular cells from the sides and these segments redivide, giving rise finally to the various tissues of the axis. In roots, daughter cells are cut off from the base of the apical cell as well as from its sides, and thus the root cap originates. The cells cut off from the sides of the pyramid divide periclinally, giving rise to the various layers of the axial structure. In roots, the first periclinal division has been said to come at once (Van Tieghem & Douliot, 1888; Chauveaud, 1903). It would seem, however, that in both root and stem it is sometimes at least preceded by a more or less radial division in each cell so that in a transverse section the tip appears divided into sextants (Russow, 1872; Johnson, 1933). In the stem of *Equisetum* it is reported also to be preceded (Vidal, 1912; Johnson, 1933) by a division parallel to that of the apical cell, a division held by Miss Johnson to form the dividing line between a node and the internode just below it.

The literature on *Lycopodium*, *Selaginella*, and *Isoëtes* reveals a different series of events. In the case of *Lycopodium*, stem apices of various species have been investigated

by a number of workers (Bruchmann, 1898; Campbell, 1918; Turner, 1924), none of whom was able to find a single pyramidal apical cell, though Bruchmann saw a prismatic 'apical initial' in the embryo just at the time when apical growth is first becoming organized. Turner described the tip with a superficial group of initials, giving rise to epidermis and cortex, with the stelar tissues arising from a deeper group. In the root, Nägeli & Leitgeb (1868) reported a wedge-shaped apical cell as in ferns, but this has not been confirmed by other workers who, working with various species, have found either four groups of initials, giving rise to root cap, dermatogen, periblem, and plerome (Bruchmann, 1874; Stokey, 1907; Campbell, 1918) or three groups, of which the outer produces both root cap and dermatogen (Strasburger, 1872*a*; Saxelby, 1908).

The situation in *Isoëtes* and *Selaginella* might be expected, from their relationship to *Lycopodium*, to resemble that described above. This is true of *Isoëtes* in a general way (Bruchmann, 1874; Hegelmaier, 1874; Farmer, 1890; Smith, 1900; Scott & Hill, 1900). In *Selaginella*, some species have the single apical cell (Pfeffer, 1871; Treub, 1876; Wand, 1914; Barclay, 1931), others have a group (Russow, 1872; Bruchmann, 1897; Wand, 1914), and stems and rhizophores of certain members of the genus have been reported to have no single apical cell at first but later to acquire one (Treub, 1876; Bruchmann, 1905).

Among the ferns, exceptions to the rule that a single apical cell is always present, are found in Marattiaceae and Osmundaceae. For Marattiaceae some earlier writers described a single apical cell (Hofmeister, 1857; Holle, 1876), but this lacks confirmation in the work of others (Russow, 1872; Schwendener, 1882; Bower, 1908). Again, some workers have described a single transient prismatic cell in the apex of very young stems (Farmer & Hill, 1902; Brebner, 1902).

In the sporeling of *Marattia alata* Miss Charles (1911) found a three-sided apical cell in both stem and root when very young. These soon changed from three-sided pyramids to four-sided prisms, and later were replaced by groups of initials. A somewhat similar procedure had earlier been reported, though in less detail, for the root of *Angiopteris evecta* (Koch, 1895). In the Osmundaceae the situation seems to be similar but less regular, some species resembling the Marattiaceae, while others are like the rest of the ferns (Bower, 1885; Campbell, 1891).

*Psilotum* and *Tmesipteris* are of interest because of their resemblance to the fossil Psilophytales, the most ancient and primitive of vascular plants yet discovered. In *Psilotum*, Solms Laubach (1884) described a single apical cell giving rise to the tissues of the underground rhizome and fruiting branch. Ford (1904) found one also in the aerial vegetative branch. This apical cell has never been seen, however, immediately after a branch forks, and is thought at this time to give way to a meristematic group, each branch of the fork later developing a new apical cell. In the adult *Tmesipteris*, as described by Holloway (1917), the single apical cell is very clearly present. In the very young sporeling, however, he was unable to say that its first-formed growing point had such a cell, and was satisfied that the second one, at the other end, did not, but arose with a group of equally important meristematic cells.

Apical growth in Spermatophyta was studied along with that in the lower plants, and is still a popular and useful subject of research. In 1865, Nicolai stated that the root growing point is a group of cells, but Nägeli & Leitgeb (1868), although they discovered an irregular meristem, looked carefully for a relationship between this and the single

apical cell of lower forms. They reported such a single cell, but a very temporary one, at the tips of rootlets in their initial stages. While a difference was recognized between the growing points of seed plants and those of horsetails and most ferns the belief in a single, transitory or obscure apical cell was defended by a number of investigators (Pringsheim, 1869; Korschelt, 1883, 1884; Klercker, 1885; Dingler, 1886; Douliot, 1890).

In 1868 Hanstein gave a fresh impetus to the research on phanerogamic growing points by returning to the policy of accurate description unhampered by the constraint of attempts to conform to earlier hypothesis. In his material he was able to trace the various tissues of the axis back to no single apical cell, but to a group of cells arranged in three layers, dermatogen giving rise to epidermis, periblem producing cortex, and plerome, the source of the central cylinder. Two years later (1870) he reported similar layers forming at the differentiation of the growing point in embryos. This concept of separate histogens in the growing point is still used to some extent, although, as will be seen, an influential group of modern investigators have found it incompatible with their discoveries on shoot apices.

In the discussions that followed, the cell arrangement in large numbers of growing points was studied intently. Gymnosperms were investigated to determine whether they exhibited conditions intermediate between those of angiosperms and pteridophytes. Strasburger (1872*b*) found in *Araucaria* the three separate histogens, in *Ephedra* a situation where periblem and plerome were not clearly divided, while protoderma (dermatogen) cells sometimes divided tangentially to add to periblem or even plerome. *Ginkgo*, *Cycas* and many conifers, he believed, presented an intermediate condition, dermatogen under certain conditions (e.g. leaf formation) contributing to the deeper tissue. He concluded that in this respect gymnosperms occupied a position intermediate between the angiosperms and *Lycopodium*.

Research on growing points took on a new lease of life, resulting in a mass of publications, some of which made noteworthy contributions to the subject. One outcome was a rather imposing accumulation of details not in entire harmony with Hanstein's scheme. In roots of some legumes plerome and periblem were not clearly separated and there was no division between periblem, dermatogen and cap (Russow, 1872; Prantl, 1874). In Gramineae and Cyperaceae dermatogen and periblem had the same initials, and the root cap came from a calyptrogen layer overlying them (Hieronymus, 1874). One investigator (Janczewski, 1874*a*, *b*) studied root tips of many plants and placed them in five groups: (1) with four separate histogens, calyptrogen, dermatogen, periblem, and plerome, (2) with dermatogen and periblem united in one, (3) with calyptrogen and dermatogen united, (4) with a transverse generative layer forming cortex, central cylinder, and the central part of the root cap, and (5) with epidermis and root cap originating in the outer part of the cortex. *Cuscuta* was found to have no root cap and no distinguishable dermatogen, periblem or plerome (Koch, 1874). Treub (1875) found three types in monocotyledons: (1) with four histogens, (2) with dermatogen eliminated, and (3) with plerome present and one other group acting as periblem, dermatogen and calyptrogen. Borzi (1887–8) reported, in addition to these three, a fourth type with but one set of initials. Eriksson (1876, 1878) found some dicotyledon roots like Hanstein's *Helianthus*, others with a plerome and a single histogen for the rest, still others with a common meristematic tissue, and a last group resembling gymnosperms. Douliot (1890, 1891), among stems of both monocotyledons and dicotyledons, found some with three initial groups and some with two.

When it is remembered that, notwithstanding such diversity in the growing points, the adult tissues comprise the normally functioning epidermis, cortex, stele, and in the root, root cap, one may feel some surprise at the persistence of a generalization that postulated a series of separate histogens, each endowed with its own discrete and limited possibilities in the way of development.

In the last few years the theory has been abandoned by a very considerable group of workers. The concept that has superseded it was first stated by Schmidt (1924). He noted that certain plants do not show the 'typical' division of the growing region into three parts, and suggested that the promeristem is differentiated into a peripheral 'tunica', one or more cells thick, and a central 'corpus'. These are not histogens in Hanstein's sense, but are distinguished from each other merely by their mode of growth. The tunica grows entirely by anticlinal divisions, except when leaves are arising from it, and is therefore in discrete layers, while the corpus cells divide in various directions and so become irregular in arrangement. The leaf primordia are thought of as folds resulting from adjustments between the methods of growth in tunica and corpus respectively. The thickness of the tunica appears to vary greatly. In *Triticum* (Rösler, 1928) and *Avena* (Kliem, 1937) it has but one layer, in *Carya* (Foster, 1935*b*), *Rhododendron* (Foster, 1937) and *Morus alba* (Cross, 1936) two are reported, in *Artemisia tridentata* (Diettert, 1938) three, in *Viburnum rufidulum* (Cross, 1937*a*) four, and in *Heracleum Sphondylium* (Majumdar, 1942) three layers with no periclinal divisions, and an additional five or six with such divisions occurring only occasionally.

It should not be concluded that the concept of separate histogens has been completely abandoned. A number of investigators, especially among those dealing with roots, still find Hanstein's nomenclature useful (e.g. Neumann, 1939; Wagner, 1939).

Those who adopted the tunica-corpus theory have benefited by their emancipation from the rigidity of the older ideas. Having freed themselves from the constraint of marshalling the apical tissue into layers on the basis of what it may later become, they are making progress by their unhampered observation of this region. Recent work on gymnosperms has been of special interest. In three cycads (Johnson, 1939; Foster, 1939*b*, 1940, 1943) the shoot apex has been found to consist of a shallow, cap-like zone of small, thin-walled cells giving rise by periclinal divisions to a rather massive central core of enlarging, vacuolating cells with thickening walls. The cells of this core divide in all directions, and neither in it nor in the apical zone has a significant preponderance of periclinal or anticlinal divisions been established. From the base of the core come off the cells that form the pith, while from its flanks and those of the apical zone is derived a peripheral tissue with both periclinal and anticlinal divisions, from which the other tissues of the shoot differentiate. In *Ginkgo* (Foster, 1938) a small, compact, apical group of cells is present instead of the apical cap, and the enlarging, vacuolating, central mother cells are in a cup-shaped mass enclosing the lower end of this apical group. It is notable that in neither case is the tissue arranged in the definite layers postulated by either of the three theories of apical growth that we have been considering. Even the cells of the outermost layer contribute by anticlinal divisions to the tissues beneath. Korody (1938), in three members of the Abietineae, found a similar diversity in planes of cell division, and suggested that the whole meristem be considered as homologous with the corpus of angiosperms, the tunica being lacking. Foster (1939*a*) deprecates this as a tendency toward the formalized attitude that limited progress under the two former theories.

Cross (1939*a*, 1939*b*, 1941, 1942, 1943*a*, 1943*b*) has investigated members of the Taxodineae and found in them a number of situations that can be considered as stages between the apex of *Ginkgo* and the typical tunica and corpus of angiosperms. In *Ephedra altissima* Desf., Gifford (1943) has reported such an intermediate stage in the form of a uniseriate tunica whose cells may rarely undergo periclinal division, and a subapical group of cells giving rise to the corpus, from which come all the tissues with the exception of epidermis.

*The leaf*

Leaves originate by the activity of meristematic cells of the outer layers on the flanks of the stem growing point. Investigation of a number of plants with a single apical cell has shown the leaf also growing from a single initial (Kny, 1875; Poirault, 1890; Bartoo, 1930; Howe, 1931; Johnson, 1933; McVeigh, 1936). This cell is developed by diagonal divisions on the part of a daughter cell of a segment of the stem apical cell. It is wedge-shaped, and cuts off daughter cells toward the ventral and dorsal sides of the developing leaf. Periclinal walls in these segments provide the primordia of the inner leaf tissues. Marginal cells divide and widen the blade.

Some work has been done on the leaf origin in pteridophytes that lack the diagrammatically clear apical initial in the shoot. In *Selaginella Martensii* the leaves are said to arise from a few external cells which divide more rapidly (Treub, 1876). The same mode of origin was noted in *Selaginella spinulosa* (Bruchmann, 1897), but a single apical cell developed later in the leaf tip. The course of development in *Lycopodium* is similar (Wigglesworth, 1907; Campbell, 1918; Turner, 1924). Three or four of the outer cell layers may take part in leaf formation. Much of the body of the leaf is produced by intercalary growth, which persists after divisions have ceased in the apical cell.

In Spermatophyta, with their several-celled shoot apices, the leaves also begin as groups of cells. Hanstein (1868) observed that in the plants he studied the leaf was a product of growth in dermatogen and periblem, an observation confirmed for a number of forms by later writers (Reinke, 1873; Herrig, 1915; Schwarz, 1927; Pottier, 1934). In other cases the plerome may take part (Jones, 1931). Some later work by those who favour the tunica-corpus concept of the growing point, gives the following results. Of forms with a one-layered tunica, *Allium porrum* (Rudiger, 1939) has corpus and tunica contributing to the leaf primordium. The same is true of *Carya Buckleyi* var. *arkansana* (Foster, 1935*b*) and of *Iris* and *Aloe* (Rudiger, 1939) with a two-layered tunica. In *Tradescantia* and *Vanilla* (Rudiger, 1939), whose tunica has three layers, and in *Viburnum rufidulum* (Cross, 1937*a*) with four, the leaf develops entirely from the tunica. The actual number of layers concerned varies somewhat. In a number of dicotyledons only one subepidermal layer takes part (Noack, 1922; Schwarz, 1927; Halmai, 1935); in others two (Massey, 1928; Zimmermann, 1928; Kühl, 1933; Weidt, 1935; Herbst, 1935). Among monocotyledons the leaf of *Triticum vulgare* is said to arise exclusively from the dermatogen (Rösler, 1928), while in *Alstroemeria aurantiaca* (Priestley, Scott & Gillett, 1935) and in *Zea mays* (Sharman, 1942) underlying layers also take part. A number of gymnosperms have been studied, and the initiation of their leaves resembles that in the angiosperms (Strasburger, 1872*b*; Korody, 1938; Cross, 1939*b*, 1941, 1942, 1943*a*; Johnson, 1939; Foster, 1939*a*). In attempting to generalize, one may say that in seed plants the early stage of leaf development consists of divisions in one, or usually more, of the outer layers at the side of the shoot apex. In most cases, periclinal divisions are

absent from the dermatogen layer, but they do occur regularly, at least in some of the gymnosperms and monocotyledons (Strasburger, 1872*b*; Korody, 1938; Rösler, 1928; Priestley, Scott & Gillett, 1935; Sharman, 1942).

In many cases it has been noted that the dome-shaped leaf primordium elongates for a time by apical growth. Sometimes the subepidermal layer provides all the initials for this growth (Noack, 1922; Schwarz, 1927; Avery, 1933), but often this is not the case (Krumbholz, 1925; Lange, 1927, 1933). In fact more than one investigator has found it impossible under normal conditions to separate derivatives of the different layers in this region (Lange, 1927; Foster, 1935*b*). Apical growth soon ceases, however, and the greater part of the length of the leaf is attained by division and enlargement of cells throughout its tissues. In *Cunninghamia lanceolata*, Cross (1942) has found a condition of general mitotic activity from the first, with no period of apical growth. In a goodly number of leaves, it has been found that during the growth period a substantial increase in thickness of petiole and midrib takes place through a cambium-like behaviour on the part of a strip of cells beneath the adaxial epidermis (Deinega, 1898; Bouygues, 1902; McCoy, 1934; Foster, 1935*a*, *b*).

The blade of the leaf is produced by growth from the edges of the adaxial side of the primordium. This may originate from strands of subepidermal cells (Gidon, 1900; Noack, 1922; Schwarz, 1927; Langdon, 1931; Avery, 1933; Johnson, 1934; Weidt, 1935; Foster, 1935*a*, *b*), or deeper cells may take part (Krumbholz, 1925; Lange, 1927), or even dermatogen cells in this region may add to the blade by periclinal divisions (Pottier, 1934; Renner, 1936; Sharman, 1942). In compound leaves the leaflets may arise in basipetal or in acropetal sequence, or the first pair may arise near the centre, the others developing in both directions. In *Carya*, according to Foster (1935*a*, *b*), their primordia arise as hemispherical protuberances on the meristematic margins of the axis.

The young leaf blade has five to eight layers of cells (Smith, 1934), and in a number of leaves these, and the adult tissues they produce, have been traced back through definite series of cell divisions to the marginal meristem (Gidon, 1900; Noack, 1922; Schwarz, 1927; Avery, 1933; Johnson, 1934), just as in earlier times the tissues of stem and root were traced back to the growing point. Marginal growth soon ceases, and the great area of the blade develops by growth and cell division throughout the tissues. The cell divisions are largely anticlinal, however, if we except those which take place in the production of procambium, so the layers are retained.

## Branching

### *Stems*

The statement is often made that branching in such primitive forms as Psilophytineae and Lycopodineae is dichotomous or 'modified dichotomous'. Such a statement is not sufficiently specific in view of the modern tendency to treat the leaves of ferns and seed plants as modified primitive branches. The need of precise comparison of the details of the origin and early development of primitive branches with those of pteropsid leaves is at once felt. In scanning the literature one is struck by the vagueness of the term dichotomy as sometimes encountered there. Some writers use it for all cases where the stem or root appears to have forked into two equal branches. Others hold that true dichotomy takes place only when the original growing point has lost its identity entirely, having undergone a median division into two equal meristems.

The branching of *Psilotum* has already been mentioned. Here, according to Ford's work (1904), the apical cell was never observed dividing in two, but it seems to disappear at the time of forking, giving way to a group of meristematic cells. The daughter branches later develop apical cells anew in their meristematic tips.

In the literature on *Lycopodium* it is sometimes difficult to decide the meaning ascribed to the term dichotomy. Stokey (1907), for example, describing *L. pithyoides*, speaks of a large stem, dichotomously branched, and Wigglesworth (1907) says that in *L. clavatum* and *L. complanatum* branching is dichotomous with one branch usually smaller. She describes a branch just dichotomized as showing at the apex 'two smaller groups of cells becoming somewhat flattened, cone-shaped structures about equal in size'. Bruchmann (1874, 1898), on the other hand, says that true dichotomy seldom takes place, and Campbell (1918) says branching is either dichotomous or monopodial and that intermediate cases occur. Where the branching is monopodial the branches arise laterally, close to the growing point and without reference to leaves, whereas in dichotomous branching there is an increase in the number of initial cells followed by forking in the apex of the plerome cylinder, and the two branches are exactly alike.

In *Selaginella* stem the prevailing mode of branching is the monopodial type described for *Lycopodium* (Treub, 1876; Bruchmann, 1897; Wand, 1914). Treub found one case of true dichotomy and thought it abnormal. Bruchmann found dichotomy in very young plants and monopodial branching later, and Wand found a gradual transition from one type to the other so that distinction was difficult.

It is noteworthy that the initial stages of monopodial branching in these stems are very similar to corresponding stages of leaf development in those macrophyllous plants not provided with a single apical cell in the growing point. The origin of the apical-celled fern leaf is less similar to the peculiar type of dichotomy described for *Psilotum*, though its derivation from such a dichotomy would not seem impossible.

Regarding the axillary branches of macrophyllous plants, there was some controversy among older workers. One group (Hofmeister, 1868; Pringsheim, 1869) held that branch primordia were produced as in *Lycopodium*, by the meristematic tissue close to the apex, while the other (Schacht, 1853; Sachs, 1870; Warming, 1872; Hagen, 1873; Vöchting, 1874, 1875) found them originating at lower positions in the bud, with a variable number of embryo leaves already formed between them and the tip. The careful developmental studies of Koch (1893) were made with a view to settling this problem, and included thirteen trees and shrubs, eight vines, five perennial herbs, seven annuals, and four water plants. His findings were in agreement with those of the second group, with a few exceptions, i.e. some flowering branches, young seedlings, and annual plants whose original growing point had deteriorated. The importance of these exceptions, where branches apparently originated in close proximity to the growing point, can hardly be assessed in the absence of further observations. It is of interest that they were invariably observed in regions pointed out by plant anatomists as conservative and likely to exhibit primitive ancestral characters (Jeffrey, 1917).

In certain of the shrubs, Koch has carefully traced the development of the branch primordia. They appear only when spring growth has begun, and the first ones are in the axils of leaves near the base of the quickening winter bud. They are thus produced by a renewal of meristematic activity on the part of small groups of cells, in tissue laid down by the growing point the year before. Division begins in the outer layer of cells, extending

rapidly to the second and third layers, and a small dome is formed. Deeper cells now divide, forming basally widening rows which press against the daughter cells of the third layer. Still deeper cells, dividing irregularly, form the connexion between the bud and the other tissues of the parent shoot. The dome-shaped outer layers retain their meristematic power, forming a growing point capable of acting in every way like that of the mother axis. Development for the time differs from that of the terminal bud in that the internodes remain short.

Koch was of the opinion that the bud primordia were composed of meristematic tissue unconnected with that of the main growing point and formed anew from cells somewhat differentiated, an opinion recently corroborated by Majumdar (1942) and Sharman (1942). Louis (1935), on the other hand, insists that the meristem of the bud is one with that of the main axis and the question of specialized tissue reverting to meristem does not arise. Whatever may be the situation with regard to axillary buds, there is no question in so far as adventitious buds are concerned. They also are exogenous, and develop much as do the normal axillary buds (Rostowzew, 1894; Bally, 1909; Carlson, 1938), but in their case the new meristem of the growing point develops from apparently typical epidermal or cortical tissue. Rhizophores of *Selaginella* develop in a similar manner (Bruchmann, 1897, 1905).

### *Roots*

In roots as in stems we have a story of dichotomous branching or near dichotomy in plants usually held to be primitive, and a production of side branches some distance back from the growing point in more advanced forms. *Isoëtes* roots are said to show true dichotomy (Bruchmann, 1874). In *Lycopodium*, Van Tieghem & Douliot (1888) and Campbell (1918) report dichotomy. Wigglesworth (1907) says the dichotomy is 'modified', one branch being at first smaller than the other, and Bruchmann (1898) reports that in larger species the branching is apparently markedly monopodial in main roots, but becomes more and more equational in the secondary branches until in the smallest ones a true dichotomy is reached. By developmental studies on sporelings he showed that the apparently monopodial branching is a modified dichotomy, the branch being initiated in the plerome region of the axis. In *Selaginella*, Bruchmann (1897) reported true dichotomy, but Treub (1876) found that the bifurcation was often due, not to a splitting of the apical cell, but the production of a new one at one side.

As to the origin of side-root branches in the higher plants, and of adventitious roots as well, Van Tieghem & Douliot (1888) reviewed the very considerable amount of earlier work, and supplemented it with observations of their own throughout all the groups. They find the branch roots originating always in differentiated tissues some distance from the growing point. In seed plants a disk-shaped group of cells in the outer layer of the pericycle divide and form a typical root growing point. In ferns and horsetails it is a single cell of the endodermis that forms the apical cell of the branch root. As a rule these roots develop opposite the protoxylem, but there are exceptions.

Adventitious roots come off from stems in much the same way. In seed plants they always come from the pericycle except in older dicotyledons, where the pericycle has developed a periderm or its cells have hardened. In such cases they may originate in phellogen, in bast parenchyma, or even in the cambium. In ferns they begin from a single epidermal cell, in *Lycopodium* and *Isoëtes* from the outer pericyclic layer, and in *Equisetum* and *Selaginella* only exogenous bud roots are formed.

In all cases, Van Tieghem & Douliot found the young endogenous roots penetrating the outer regions of the parent root or stem by digesting the cells. For the most part a digestive sac was formed about the young tip by the endodermis of the parent, but in other cases the endodermis itself was dissolved by digestive action on the part of the tip.

Some exceptions to the generalizations of Van Tieghem & Douliot have been noted. Pond's experimental investigation (1908) failed to discover any evidence of cell digestion and indicated merely mechanical penetration, but Smith (1936), in *Begonia*, noted a pocket formed by dissolution of cortical cells even before the root histogens had differentiated. In *Marattia alata*, the adventitious roots were found originating in the meristematic region of the stem (Charles, 1911). In *Ceratopteris thalictroides*, they are reported as arising from the stem hypodermis (Howe, 1931). Despite such individual disagreements, the work of Van Tieghem & Douliot still stands as a most useful morphological work on the origin of endogenous organs in general.

## PRIMARY VASCULAR TISSUES

Numbers of workers, using seed plants for the most part, have interested themselves in searching out the order of development in the primary vascular system. Miss Esau's recent comprehensive review of this work (1943) has proved most useful, and has been utilized freely in the preparation of the present brief sketch.

### *Residual meristem and procambium*

Nägeli's (1858) belief in the development of separate 'procambial strands' directly from the meristematic tip of all stems and the development from these of a 'cambium ring' did not long remain unchallenged. Even before him, Karsten (1847) found an unbroken ring or cylinder of tissue extending down from the 'Urmeristem' between cortex and pith, in which the vascular bundles developed, a finding corroborated for certain plants by others (Schacht, 1856; von Mohl, 1858) and later (Sanio, 1863; Koch, 1891; Bouygues, 1916) extended to most if not all seed plants, with certain modifications. Koch (1891), for example, discovered that in gymnosperms the ring included the cortex, and corroborated Sanio's statement that sometimes the longitudinal cell divisions that produce the elongated, narrow cells of the procambial strands occur very close to the apex, before the ring has been delimited. The concept of a ring of meristematic tissue delimited below the growing point by the beginning of differentiation, in which ring the procambium is developed, is substantiated by some researches with modern technique. Helm (1931) found that, when sections of fresh tissue were treated with hydrogen peroxide, bubbles were given off by cells differentiating into pith and cortex, but none were evolved from apical meristem or from the meristematic ring or the procambium strands. By this method he was able to recognize the ring even in cases where ordinary staining tests failed to separate it from adjacent tissues and found that it extended above the youngest leaf primordia. Cytologically its cells resembled those of the apical meristem, of which he considered it an undifferentiated remainder.

Louis (1935) differed from Helm, believing that the meristematic ring, which he called prodesmogen, was not to be seen above the uppermost leaf bases. According to his view, the young leaf, when it joins the stem, retains a plate of prodesmogen extending across it from side to side, while parenchyma develops above and below the plate. The

cortical parenchyma of the stem differentiates downward from the dorsal parenchyma of the leafbase, and thus the outer limits of the meristematic ring in the stem come into being only in connexion with the leaf rudiments, though pith may develop and determine its inner limit above this level. In his work Louis differed from Helm in that he made no use of vital tests.

Kaplan (1936, 1937) used the name 'residual meristem' to represent the tissue we have been considering. He investigated it in many forms and found that in the stem it may form a ring with cortex outside and pith within, may include all tissues outside the pith, or may take the form of a solid core, or of several concentric rings. In the young leaf it forms a median plate or band reaching from side to side and extending from the tip through the base to connect with the similar tissue in the axis.

This tissue is traversed by the strands of procambium from which will develop primary wood and bast. The procambial cells are formed from the normal meristematic cells by repeated divisions parallel to the axis of the strand, so that from the first they are narrow and long, a peculiarity later accentuated by further elongation. Concerning the position where procambium first appears there has been some disagreement, as is to be expected when one remembers that divisions parallel to the axis are not limited to procambium initials, but may occur, along with divisions in other directions, in the surrounding cells. Helm (1931, 1937), Louis (1935) and Kaplan (1936, 1937) found the procambium differentiating in the form of strands in the meristematic ring below the growing point, the strands in so far as seed plants are concerned being related always to leaf primordia. Kostytschew (1922, 1924) had, it is true, stated that usually the procambium appeared first as a solid ring, later to be broken up into strands, but, as has lately been pointed out by Esau (1943), he failed to distinguish clearly between the procambium and the meristematic zone that preceded it. A number of modern workers on stem growing points (Schmidt, 1924; Zimmermann, 1928; Foster, 1935*b*; Cross, 1936; Boke, 1940; Cross & Johnson, 1941) find procambium extending very close to the growing point, and throw doubt on the concept of the meristematic ring as an intermediate step between apical meristem and procambium. It may be found that generally the procambial tissue begins differentiation at least as early as do cortex and pith, in which case the residual meristem would be properly considered merely as undifferentiated tissue surrounding the strands, and in no sense as a stage in their initiation.

The procambial strands whose development has been studied in seed plants are leaf-trace strands, but Kaplan (1937) found that in protostelic pteridophytes the procambium formed a solid plug, and in most ferns the residual meristem of the axis changed uniformly to procambium. There has been a difference of opinion as to the direction of development in the strands. Earlier writers often fail to differentiate clearly between procambial development and differentiation of wood and bast, but even in more modern times, when the distinction has been clearly made, the discrepancy has persisted. Flot (1905, 1906, 1907), in a somewhat general survey of dicotyledons, found the procambial development beginning at the base of the primordium of a leaf and proceeding basipetally into the stem, a condition in agreement with that stated much earlier for monocotyledons by Guillaud (1878) who found it developing from the leaf base down into the stem on one hand and out into the leaf on the other. A number of workers report similar findings (Herrig, 1915; Bugnon, 1921; Lange, 1927; Langdon, 1927; Yarbrough, 1934; Foster, 1935*a*, *b*; Cross, 1936, 1937*a*, *b*) and numerous others have assumed the correctness of

these discoveries. On the other hand, Koch (1891, 1893) stated that in gymnosperms and dicotyledons development proceeded acropetally up the stem towards the leaf primordia and into the leaves, and a number of later workers are in agreement with him. Priestley, Scott & Gillet (1935) in *Alstroemeria* describe the procambium strand as developing acropetally from its origin in connexion with the base of an older leaf, through sixteen internodes to the base of the primordium whose leaf trace it is to supply. Boke (1940) in the phyllode of *Acacia* was able to find no break in the continuity of the procambium strands at any time, and he also (1941) found that in certain Cactaceae the development was acropetal. Cross & Johnson (1941) found acropetal development from stem to leaf in *Vinca rosea*, and Cross (1942) concluded that the same was so for *Cunninghamia lanceolata*. Esau (1943) states her inability to find discontinuity in the procambium in any of the shoot apices that have figured in her researches. Clearly the problem, important as it is to the student of causal morphology and to all who would understand the fundamental scheme of the plant body, requires further research for its complete elucidation. The differences of opinion are explainable, considering the minuteness of the structures and the shortness of the internodes in the young bud at this stage of development.

In the growing leaf there is agreement that the procambium of the main veins develops acropetally through the blades, giving off branches. In monocotyledons secondary longitudinal and cross-veins may develop back from the tip after the main ones have reached it (Priestley, Scott & Gillett, 1935; Sharman, 1942). For a time the procambial strand increases in thickness due to divisions in the original procambial cells and to additions from surrounding tissues (Thoday, 1922; Foster, 1935*a*, *b*; Esau, 1936*a*, 1938*a*).

### *Xylem in shoots and leaves*

Differentiated xylem elements are easily recognizable, and the order of their development has been established in a large number of plants. Among the earliest writers there was discussion as to whether it was basipetal or acropetal, but there is agreement among investigators of the last sixty years that at least in dicotyledons and gymnosperms the differentiation begins normally in the trace near the base of the leaf or in a vein and develops basipetally in the stem to unite with the wood of older traces (Lignier, 1890; Trécul, 1891; Bugnon, 1922; Langdon, 1927; Priestley & Swingle, 1929; Griffiths & Malins, 1930; Langdon, 1931; Priestley, Scott & Gillett, 1935; Priestley & Scott, 1936*a*; Hasselberg, 1937; Gráf, 1937–8; Esau, 1938*a*; Crafts, 1943). A discrepancy is reported by Trécul (1891) in the case of *Primula*. Here he found that in the most vigorous buds the condition was as described above, but in weak ones, where leaves developed very slowly, development was acropetal from the point of insertion on an older leaf trace through the stem up into the new leaf. There were also shoots of medium strength where the wood differentiated from both directions, the two ends meeting at some intermediate point. Trécul studied numerous other plants where the development was invariably basipetal in the stem, but he often found a short vessel protruding from the mature bundle ready to join the new trace approaching from above. In monocotyledons, the evidence covers fewer forms, and more investigation is desirable. Trécul found in the Gramineae (1878) and in a number of other monocotyledons (1880) that, in the main vein, differentiation was acropetal from the stem through sheath and leaf, while subsidiary strands developed back from the tip and up from the base, the two ends fusing where they met. Reference to the basipetal development of subsidiary veins is also seen elsewhere (Prantl,

1883; Schuster, 1910; Scott & Priestley, 1925). Scott & Priestley distinguished in *Tradescantia* stem four sets of bundles, medullary, perimedullary, cortical, and peripheral, thus agreeing with the earlier work of Gravis (1898–9). All the bundles fuse at the nodes, the two central sets forming a mass, and the peripheral ones a ring connected with the central mass by anastomosing strands. The authors do not state in so many words whether differentiation of the main leaf traces is acropetal or basipetal in the stem. They note, however, that the medullary bundles, after passing up through one internode, enter the nodal mass of tracheids and come out as perimedullary bundles which pass into the leaf at the next node above, constituting its primary longitudinal veins, and they further state that 'at any one level of the stem, the perimedullary bundles are the first to show lignified xylem'. Thus is indicated a basipetal development of xylem in these main veins from the vicinity of the leaf base to the medullary bundles in the second internode below, a finding in agreement with that of Nägeli (1858) in *Chamaedorea* and *Cordyline*. The authors observed the development of these main veins into the leaf. The median one developed most rapidly, and when its xylem neared the tip of the leaf, tracheids were formed on either side and from them a pair of marginal veins developed basipetally, connecting up with the ends of the more slowly developing lateral veins. In these, metaxylem then began to form, its development proceeding basipetally. Transverse veins and subsidiary longitudinal ones now developed from the tip backward, the longitudinal ones entering the stem and developing basipetally in it as cortical bundles, which either ended freely in the cortex of the internode or continued down to connect with the wood of the node beneath. At the same time the peripheral bundles developed basipetally from one node to the other, and the rapid intercalary growth of the internode ruptured the wood of the medullary and perimedullary bundles.

The development of xylem in the leaf blades of dicotyledons has still to be considered. In some cases it is reported to resemble that in monocotyledons in that primary veins develop acropetally and secondary ones basipetally (de Lanessan, 1876, 1877; Prantl, 1883; Schuster, 1910), but this is not the general condition. A centre in the leaf or leaf trace, from which development spreads to stem and leaf, has already been mentioned. The position of this centre may be at almost any point in the procambium of leaf or leaf base, or there may be several in one leaf (Trécul, 1891), development from each proceeding in both directions so that all veins finally become continuous and united with the vascular system of the plant.

### *Phloem in shoots and leaves*

The course of phloem differentiation in shoots and leaves has received little attention in comparison with that devoted to the xylem. It is more difficult to distinguish from procambium than is the xylem, and this may account for the relatively few precise observations on its development. A few (Guillaud, 1878; Lignier, 1890; Priestley & Swingle, 1929; Hasselberg, 1937) have considered it as developing by the same steps as seen in the xylem, but the weight of the evidence is for acropetal development in continuity with the mature bast of the axis (Griffiths & Malins, 1930; Chang, 1935; Esau, 1938*a*, 1939; Sharman, 1942). In general the phloem develops earlier than the xylem (Sanio, 1863, 1864; Russow, 1872; Schmitz, 1874; Griffiths & Malins, 1930; Priestley, Scott & Gillett, 1935; Esau, 1938*a*), but there is evidence that internal phloem when present is an exception to this rule (Weiss, 1883; Baranetzky, 1897; Léger, 1897–8; Avery, 1933; Esau, 1938*a*).

### *Vascularization of buds*

The vascularization of axillary buds still provides a field for useful research. If the condition found by Louis (1935) is the normal one, the prodesmogen (residual meristem) of the bud forms a unit with that of the main axis, and one might look for a regular development of procambium strands and vascular bundles joining the leaves of the bud with the stele of the stem in normal fashion. If, on the other hand, Koch's statement (1893) of the isolation of the meristematic bud primordia should prove to describe the general situation, new problems would present themselves, for the vascular bundles joining bud to stem would have to come from cells at least partly differentiated. Koch reported in such cases, that the procambium developed anew from parenchyma, first in the middle region and later by extension in both directions.

In the case of the adventitious buds so important in plant propagation, there is no question that the vascularization often passes through differentiated parenchyma tissue. In 1887 Beijerinck noted that such buds on the roots of certain woody plants made connexion by centripetal growth of traces. Simon (1908), in buds formed from callus on woody cuttings, also found this centripetal differentiation, but went more into detail. According to his account some callus cells just beneath the bud primordium divide to form a procambium in which tracheids differentiate. The row of tracheids extends itself towards the stele of the cutting, the inner ones developing from undivided callus cells. Strands also develop outwards from the vascular cylinder of the stem, their xylem meeting that from the bud. When the xylem connexion is complete, further meristem develops and completes the bundle. Inward development of procambium and xylem has also been observed in other cases (Boodle, 1920; Crooks, 1933). On the other hand, isolated beginnings of xylem with one end developing towards the bud and the other towards the stele, have been observed (Priestley & Swingle, 1929; Naylor, 1941). Further investigation is desirable. In particular, critical examination of the phloem differentiation in a number of forms would be of assistance in filling out the picture.

### *Vascularization of roots*

In roots as in stems there is either a solid core or a ring of meristematic tissue behind the growing point. Russow (1872) called it 'desmogen', De Bary (1884), the 'initial strand', and Haberlandt (1904), 'procambium'. Others prefer to consider it residual meristem (Hof, 1898; Kaplan, 1937). They spoke of it as a solid plug in the ferns they studied. In the Angiosperms, *Vitis*, *Cissus* and *Vanilla*, Kaplan found a meristem ring in which separate, alternating, procambium groups developed into wood and bast respectively. With regard to the direction in which development proceeds, there is no difference of opinion so far as the root is concerned. All agree that both xylem and phloem develop acropetally, always in contact with the more mature tissues.

## CAMBIA AND SECONDARY TISSUES

### *Fascicular and interfascicular cambium*

It has already been observed that the procambium strands normally increase in thickness, partly at least, by longitudinal division of the procambial cells. In many stems these divisions are predominantly in a plane tangential to the axis, so that most cells of the so-called primary bundle have the radial arrangement characteristic of secondary tissues.

Esau (1943) has listed many references to this condition, including members of all major groups of vascular plants. These divisions may continue in the median part of the bundle after protoxylem and protophloem have matured at its edges (Esau, 1936*a*, 1938*a*). The resulting controversy as to the boundary line between primary and secondary tissues is a question of terminology with which we need not concern ourselves. It is sufficient to note that the distinction is not sharp, and that secondary growth as seen in dicotyledons and gymnosperms may be considered the further development of a tendency to be found normally in the primary tissue of the procambial strands. In stems with interfascicular cambium, this tissue is said to develop across from the edges of the fascicular (Messeri, 1936). Kostytschew (1922, 1924), from a study of the stems of nearly 150 species of dicotyledons, held that all forms with a solid vascular cylinder had a solid ring of procambium in which the cambium developed. Those with isolated procambium strands had, he said, distinct vascular bundles, the interfascicular cambium in such cases being able to produce only parenchyma cells. Carstens (1931) was in agreement with Kostytschew, and extended the thesis to gymnosperms. It has since been found, however, that in certain cases Kostytschew was mistaken in believing that no wood was produced by the interfascicular cambium (Duncker, 1936; Sárkány, 1936; Gráf, 1937–8). Should his thesis prove correct to the extent that there is a markedly greater aptitude for the production of vascular tissue in a cambium produced directly from the less specialized cells of procambium or residual meristem, the fact is of interest to students of developmental morphology, though it must be remembered that attainment of the cytological characteristics of parenchyma does not always preclude the possibility of producing vascular tissue. In *Ginkgo* and the cycads it has already been shown that the vascular bundles, with most of the rest of the shoot, are derived from a group of vacuolated, parenchyma-like cells in the apex, and the production of wood and bast from more or less mature tissues in connexion with adventitious buds is well known. In roots, the whole of the cambium is interfascicular, the wood and bast being in separate, alternate bundles, and the cambium differentiating between them.

The cambium, as seen in gymnosperms, dicotyledons and some vascular cryptogams, consists of two types of cell. The fusiform cells give rise to tracheids, wood fibres or vessel cells on the one hand, and sieve tubes or bast fibres on the other, while the cells that produce the rays are short, and more or less rectangular. The longitudinal division of the fusiform initials is interesting, and is described in detail by Bailey (1919, 1920*b*, *c*). The mitotic spindle is diagonally placed near the centre of the cell, and, as its fibres disappear in the formation of the cell plate, new peripheral ones form successively in the cytoplasm, the plate thus extending up and down the cell until it reaches the ends. Since the divisions are tangential the result is the addition of new cells to the radial rows of secondary wood and bast. The rays are similarly added to by normal, tangential divisions of the ray initials. A new uniseriate ray may originate by horizontal divisions in a fusiform cambial cell, changing it to a series of ray initials, and may increase in size by the further division of these initials or by the addition of new ones cut off from adjacent fusiform cells (Chattaway, 1933).

Some observation has been made of the development of annual rings in the wood of trees. The activity that leads to the production of spring wood spreads down branch and stem from the elongating bud (Cockerham, 1930; Schmidt, 1933; Wight, 1933; Wray, 1934; Brown, 1935) and extends to the basal part of the root (Cockerham, 1930). It is

stated by Priestley & Scott (1936*b*) that just as spring wood formation is initiated in the vascular differentiation of the leaves of the extending shoot, so summer wood development connects with the developing bundles of the bud scales.

A cylinder of thin-walled tissue produced by late cambial divisions remains undifferentiated through the winter, and it has been found in *Acer pseudo-platanus* that the first spring tracheae (Cockerham, 1930) and the first sieve tubes (Elliott, 1935) develop from these cells, before cell division begins in spring. No bast is produced while wood is forming, but smaller, summer sieve tubes are formed later, their production continuing until leaf fall. In the ash it has been found (Gill, 1932) that the spring and summer sieve tubes differ from the autumn ones in their large size and the fact that each usually has but one companion cell instead of two.

Bailey (1923*a*) has studied the mode of increase in girth of the cambium as secondary wood is added to the stem. He found that in some highly specialized dicotyledons with short fusiform initials this increase is brought about for the most part by radial longitudinal cell divisions. In other plants the result is due chiefly to divisions transverse or nearly so, and subsequent elongation of the daughter cells, with gliding growth. Klinken (1914) had already discovered this gliding growth in the cambium of *Taxus*.

### *Periderm development*

In the formation of periderm the phellogen or cork cambium is a single layer of cells dividing periclinally, and may be derived from any tissue, from the epidermis to the secondary phloem (Sanio, 1860; Douliot, 1888*a*, *b*, 1889). Here we have an undoubted case of meristem, usually, though not always, a series of successive meristems, formed from differentiated tissue. The tangential divisions of this meristem cut off more or less cuboidal cells, phelloderm towards the centre, and cork, with its walls becoming suberized, on the outside.

### *Secondary growth in monocotyledons*

There is a considerable literature on secondary growth in monocotyledons. Radial arrangement is often seen in their cauline bundles (Sanio, 1863; Andersson, 1889; Gravis, 1898–9; Decrock, 1905; Chrysler, 1906; Queva, 1907; Arber, 1917, 1918, 1919, 1922; Dauphiné, 1917; Gatin, 1920; Joshi, 1939), and is interpreted as indicating secondary growth from an ephemeral cambium. The second, less transitory type of secondary growth, noted as long ago as 1865 by Millardet, is much less common, but is found in numbers of Liliflorae and some other monocotyledons. The cylinder of secondary tissue is composed of numerous vascular bundles embedded in secondary parenchyma, the radial arrangement of which is considerably distorted in their neighbourhood. The secondary vascular bundles are collateral in some forms, amphivasal in others. Growth rings have been found in these secondary tissues in a number of species (Hausmann, 1908; Lindinger, 1909*a*, *b*; Chamberlain, 1921; Cheadle, 1937). Such zonation, when present, is due to variations in size or number of vascular bundles, or in size or wall thickness of secondary parenchyma cells, or any combination of these causes. This was noted by Cheadle (1937) along with evidence that the type of ring is in some cases a specific, but not a generic character.

In the seedling plant secondary growth occurs first in the hypocotyl (Wright, 1901; Hausmann, 1908) extending into the stem, and in some cases into the root, as the plant grows.

There has been some difference of opinion as to the true nature of the cambium. Millardet (1865) found a cambium three or four layers thick. Röseler (1889) said a regular cambium layer was absent and that meristematic cells were scattered throughout the secondary thickening. Schoute (1902) perhaps discovered the reason for this discrepancy when he observed that although at first the cambium is irregular, one cell dividing a few times and then losing all meristematic power as other neighbouring cells take their turn, this condition finally gives way to a regular, permanent cambium. Later workers (Hausmann, 1908; Lindinger, 1909*a*) are in agreement with Schoute.

There has been some disagreement as to the position of the cambium, whether in pericycle or cortex, accounted for by the fact that in the stem tip there is not a clear demarcation between these two tissues. In the root, such a difficulty does not exist, and Cheadle's (1937) photographs of early stages of secondary growth in roots of *Dracaena* show the location of the cambium clearly within the well-differentiated endodermis.

In the secondary tissue, the development of each vascular bundle begins with longitudinal divisions in a single strand of cells (Scott & Brebner, 1893; Hausmann, 1908; Chamberlain, 1921; Cheadle, 1937). One or two adjacent cells subsequently divide in a similar fashion, and a bundle results identical in appearance with the procambial strand of primary tissues. From this the wood, bast and parenchyma of the fibro-vascular bundle differentiate. The cambial cells and the daughter cells derived from them are comparatively short, and the elongation of developing tracheids to fifteen to forty times their original length (Cheadle, 1937) is a gliding growth. The number of tracheids seen in the transverse section of a mature bundle is thus much greater than the number of tracheid primordia in any one tier of cells in the young strand. Bast elements and wood parenchyma cells elongate little if at all.

It has been observed by Scott & Brebner (1893) and Cheadle (1937) that young leaf traces, beginning as primary bundles, are completed by the secondary meristem, and thus the primary and secondary conducting tissues are united.

## Individual specialized cells

### *Parenchyma*

The parenchyma cell matures with perhaps less specialization than any other. Even so, its development presents a number of important stages, of which a thorough understanding is desirable. Developmental stages between a typical meristematic cell of the shoot growing point and a mature parenchyma cell have been described by Schüepp (1926) and Priestley (1929).

The small, twelve- or fourteen-sided meristematic cell has a comparatively large nucleus, and dense cytoplasm with no visible vacuoles. Its thin wall is plastic, not elastic, and often takes up protoplasmic stains. When the cell is in an active state, protein synthesis is notably to the fore, and regular cell divisions are occurring.

In the next stage of development vacuoles begin to form, and at the same time the metabolism of the cell changes so that protein synthesis becomes less rapid, being partially replaced by carbohydrate metabolism, as evidenced by the more rapid deposition of cellulose on the wall. Often in the early stages of this development much starch is to be seen in the cells (Swarbrick, 1927; Priestley, 1929), but this soon becomes hydrolysed. The cell gradually increases in size as water is added osmotically to the vacuole, and it

begins to round up, leaving spaces filled with fluid at the corners between it and its neighbours. During this stage cell divisions still take place, but less frequently than before.

In time cell division ceases, and the cell enters a comparatively brief phase of rapid expansion by vacuolation. The beginning of this phase coincides, according to Priestley's observations, with the entrance of air into the intercellular spaces of the shoot. In most tissues the enlargement is very largely an elongation process, but this is by no means always the case, the swelling reaching a climax of irregularity in the spongy mesophyll of leaves and certain other tissues with large air spaces. The stage of rapid expansion is soon finished. The pectic constituents of middle lamella and primary wall become hardened as they change to calcium pectate, and the cell, its plastids and other inclusions having matured in the meantime, becomes an adult parenchyma cell.

### *Collenchyma*

Cells of this tissue, with their peculiar, unevenly thickened walls, develop from parenchyma cells. Van Wisselingh (1882) emphasized this. In his material, by the time collenchyma differentiation began, the parenchyma was well developed with air spaces among the cells, and in the neighbouring vascular bundles the procambium stage had been attained. Here and there a parenchyma cell underwent a radial or a tangential division, and the walls began to thicken irregularly. In some cases the cells retained approximately their original dimensions. In others they elongated greatly and even became divided by thin cross-partitions. Ambronn (1881) had already noted that this growth in length continued after the secondary thickening had taken place. Pits formed in the thickened walls, circular, oval, or more elongated. As the cell wall thickened at the corners, the intercellular spaces often became completely filled, though in plants where the thickening was not so pronounced, small spaces, circular or even angular in outline, were left.

Esau (1936*a*) observed the development of collenchyma fibres in celery. Here also the parenchyma cells from which the fibres originated had developed vacuoles and intercellular spaces, but in this case repeated radial and tangential divisions produced strands resembling procambium, and from these strands bundles of collenchyma developed. The cells elongated greatly and became cut up by very thin cross-partitions.

The middle lamella of collenchyma cells has been identified as of a pectic nature (Anderson, 1927*b*). Giltay (1882) found that when this lamella was dissolved by chromic acid two layers of doubly refractive substance were left. The objection to considering this substance pure cellulose lies in its ability to absorb and hold large quantities of water. This capacity, observed by many earlier morphologists, was confirmed by Cohn (1892), who made careful quantitative tests and showed it to be much greater than that of the walls of various wood cells and fibres. When dehydrated the contraction radially was 27%, tangentially 10%, and longitudinally $\frac{1}{2}$–$\frac{3}{4}$%.

Giltay showed layering in the secondary part of the thickened walls. Anderson made microchemical tests and held that the walls were composed of numerous, fine, alternating lamellae of cellulose and pectin, the latter accounting for the great swelling and water absorption. He made no observations on the laying down of these layers during development.

### *Epidermis*

The developmental stages we have noted in parenchyma are to be observed also in epidermal cells, but with some modifications and additions.

The epidermis of most shoots and of some roots develops a cuticle. Lee & Priestley (1924) saw the cuticle arising simply by a migration of soluble fatty compounds from interior cells of the shoot near the growing point through the cell walls to the outer epidermal surface, where they became condensed and oxidized to form the resistant layer of cutin. The investigations of other workers, however (Frey, 1926; Anderson, 1928, 1934), suggest a more complex structure than the above would indicate, and in the light of their work a careful developmental study should be useful. Frey's work with polarized light showed in the epidermis of leaves of *Clivia nobilis* an outer wall with a definite isotropic layer near its centre and differing intensities of double refraction as one passes inward and outward from this. In the petiole of *Acuba japonica* he found another condition unexplained by Lee & Priestley's hypothesis, namely, the presence of isolated plates of cutinized cellulose over the cells. Anderson's microchemical findings on *Clivia* were in agreement with Frey's results. He found the central, isotropic layer to be composed of pectic material. Inside this, the proportion of pectose became less and less as one approached the protoplasm of the cell, its place being taken by increased amounts of cellulose. Outside the isotropic layer, the wall was composed of two zones. The inner contained pectic material, cellulose, and cutin, the latter substance increasing at the expense of the other two as the outer limit was approached. The outer zone contained only cellulose and cutin, the cellulose decreasing toward the outside, and outside this second zone was a cuticle, composed of pure cutin. One peculiar type of cell had cutin deposited also in a layer adjacent to the protoplasm, an uncutinized zone lying between it and the outer layer of cuticle (Anderson, 1934). Moreover, Anderson says 'the cellulose and pectic material in the outer wall of each epidermal cell are separated from other cells by vertical partitions of cutin that contain little or no cellulose'.

Martens (1933, 1934*a*, *b*) developed a conflicting view in his observations of cuticle development on certain flower petals, where he conceived this layer to be laid down very rapidly, in irregular folds, and not in close contact with the cellulose wall beneath. The folds later became effaced or reoriented by stretching as the cell grew. Corroborative reports on other material have not been found.

In *Viscum album*, a perennial dicotyledon without periderm, a peculiar situation arises (Damm, 1901). The epidermal cell develops a cuticle 4$\mu$ thick, after which the outer wall thickens greatly and develops in three layers—cellulose, a cuticularized layer (of cellulose and cutin) and cuticle. The cuticle extends in along the radial walls. Later, cells of epidermis and cortex become completely enclosed and cut off by cuticle, subsequently dying and forming characteristic brown masses.

The literature contains a number of references to the development of the numerous varieties of hair that appear on the epidermis. Hirsch (1900) reviewed earlier literature on the subject, finding elongation by basipetal, acropetal and intercalary growth, the third type sometimes accompanied by the first or second, and since that time stages of development in the hairs of a few species have been noted (Windel, 1916; Cooper, 1932; Franz, 1935).

The cotton hair has claimed the attention of investigators because of its economic

importance. It begins (Balls, 1915; Anderson & Kerr, 1938) as a slight swelling on the outer wall of an epidermal cell, and elongates for 15–20 days, forming a delicate tube. The primary wall consists of some pectic substance, with a small amount of cellulose. The cellulose is arranged in two systems of spiral strands, and also probably a transverse system (Anderson & Kerr, 1938). The secondary wall, which begins to form after elongation is complete, is laid down in layers, one layer for each day (Balls, 1919; Kerr, 1937). The layering has been shown to be due to changes in temperature and light intensity (Kerr, 1937; Anderson & Moore, 1937). The layers are composed of cellulose arranged in spiral strands. The direction of the spiral can be reversed fifty times or more in the length of a hair, and the points of reversal frequently do not concide in successive layers. Farr & Eckerson (1934*a*, *b*) saw ellipsoidal particles in the cytoplasm of developing hairs, and interpreted them as bits of cellulose being added to the wall. This interpretation has been challenged by Anderson & Kerr (1938).

Root hairs, like cotton hairs, begin as swellings on epidermal cells, and have during growth a primary wall with a pectic and cellulose layer (Roberts, 1916; Hopmann, 1930; Cormack, 1935). There is evidence that the wall of the growing hair is softer at the tip than elsewhere, a condition thought by Ziegenspeck (1920) to be brought about by the presence of amyloid in place of cellulose in this region. His results were not confirmed, however, by other workers. On the subject of growth of root hairs, experimental work of some interest has been done and will be reviewed under another heading.

### *Endodermis*

The endodermal cell can be distinguished from a parenchyma cell at an early stage owing to the development of a continuous Casparian strip in its radial and horizontal walls. This strip was figured many times by older writers on growing points, who made use of it freely in their identification of tissues in early stages.

Kroemer (1903) recognized three stages in the development of a typical endodermal cell in angiosperms, in addition to the initial stage when it could not be distinguished from a meristematic cell. In the primary stage, the Casparian strip was laid down; in the secondary, a suberin layer was deposited over the inner surface of the cell wall; and in the tertiary, inner layers, mainly of cellulose, were added within the suberin lamella. These stages are not all invariably passed through, however. In most pteridophyte roots, development ceases with the primary stage (Rumpf, 1904; Mager, 1907; Bäsecke, 1908; Priestley & Radcliffe, 1924), and while in those of leptosporangiate ferns it reaches the secondary (Rumpf, 1904), no plant below the Spermatophyta has been reported to attain the tertiary.

The Casparian strip is laid down at an early stage of development, while the endodermal cell walls still display the properties characteristic of meristem (Priestley & North, 1922). It is apparently confined to the middle lamella, which, according to these authors, becomes thickened and impregnated with a substance that reacts as lignin to microchemical tests, and has also some properties characteristic of fatty substances.

The suberin lamella of the secondary stage differs from the suberin of normal cork in that it lacks recognizable phellonic acid. In some plants it is present only on the inner tangential wall of the cell, in others it lines the entire wall, and in still others, as mentioned above, it is absent. When pits are present in the suberized wall, the suberin extends over the pit membranes. This renders the endodermis relatively impermeable to water and

solutes, but in many cases passage cells are left unsuberized, and through them osmosis can take place.

### *Tracheids*

The bibliography of the development of tracheids is comparatively short. Papers dealing with the nature of tracheid walls are numerous, but the majority of modern workers have not approached the problem from the developmental angle. Some earlier investigators did so, however, drawing some conclusions that more refined instruments and methods have shown to be erroneous, and others that still stand. Sanio (1873) noted that the cambium cells in the pine and the young tracheids cut off from them had primary walls that became bright blue in chloriodide of zinc, and were held together by a middle lamella that we now know to be pectic in nature. The tracheids enlarged, stretching their radial walls, and, he believed, resorbing the substance of the radial middle lamella. Toward the end of this resorption period began the gradual deposition of the distinct secondary wall. Successive lignification of primary and secondary walls followed. The tertiary wall was a thin layer laid down inside the secondary, from which it was distinct optically and chemically, but not genetically. Sanio found the various wall layers distinguishable at all times by their differential reactions with chloriodide of zinc. He believed the primary wall continued to increase in thickness after the secondary had been formed between it and the cell protoplasm, and on this basis favoured the hypothesis of cell wall growth by intussusception.

On the form and development of bordered pits in the tracheid walls, a number of conflicting accounts were given (Trécul, 1854; Dippel, 1860; Hartig, 1862; Sanio, 1873; Mikosch, 1881). Of these, Sanio's description is in best agreement with what one observes in the vicinity of the cambium with the more refined instruments now available. The pits, as described and figured by Sanio, first appear as thin spots on the radial wall. They become thinner by stretching. Later the thickened torus forms in the centre. This thin 'primordial pit' reaches a considerable size, and may have one, two, or more tori and bordered pits formed on it. The bordered pit is carefully described, and stages in the growth and arching over of the secondary wall are illustrated. The thickened rim of the primordial pit was described by him for the first time and has been named the 'rim of Sanio'.

It is usual for tracheids to increase in length as well as in thickness during development. In the case of primary wood this increase is accompanied by expansion of the surrounding tissues in the same direction, but in secondary tissues, produced after general elongation has ceased, every increase in length of a cell means a corresponding amount of gliding growth. In *Ginkgo* and the conifers the length of mature tracheids is usually only slightly greater than that of the cambium cells from which they arise. Fibre tracheids in dicotyledons are, on the other hand, considerably longer (Bailey, 1920*a*).

In monocotyledons with secondary vascular bundles the tracheids may reach a length, according to Kny's (1886) and Cheadle's (1937) measurements, thirty or forty times as great as that of the young cambiform cells from which they arise. Kny believed that one such tracheid arose from a row of cambiform initials by the dissolution of their end walls. Röseler (1889) failed to find any evidence of such a process, but contended that each tracheid came from the growth of an individual cell, and his conclusions have been confirmed by the work of Scott & Brebner (1893) and Cheadle (1937).

*Tracheae*

Two main characters distinguish tracheae from tracheids, i.e. their perforated ends, and their usually greater diameter. Some attention has been devoted to their development in secondary wood.

The increased diameter is due to expansion occurring promptly after the trachea is cut off from its mother cell in the cambium. Bailey (1920*a*) has shown that, while the fibre tracheids in angiosperms have retained the ancestral ability to increase in length, the tracheae have exchanged it, more or less, for a tendency to increase in girth, so that in primitive angiosperms the tracheae are little longer and somewhat wider than the cambium cells, while in the more specialized, they increase enormously in girth and not at all in length. The rapid growth in diameter often results in extreme distortion and disarrangement of the surrounding cells while their walls are still plastic (Velten, 1875; Zimmermann, 1922; Priestley, Scott & Malins, 1935; Esau, 1936*a*; Esau & Hewitt, 1940).

The perforation of tracheal end walls to form the continuous tubes we speak of as vessels was established in the earlier half of the last century (Treviranus, 1835; Mirbel, 1837; von Mohl, 1845*a*, *b*; Crüger, 1855). Many investigators have agreed that the dissolution of these walls takes place late in the development of the trachea, after the secondary thickenings have developed on the sides and on any part of the end wall that is to be permanent (Strasburger, 1882; De Bary, 1884; Lange, 1891; Smolák, 1904; Němec, 1910; Flach, 1924; Duerden, 1934; Esau, 1936*b*; Esau & Hewitt, 1940), but Priestley, Scott & Malins (1935) found them disappearing at a much earlier stage, while the side walls were still very thin, and were presumably of the same nature as those at the ends. They thought of the end wall as being broken by the increase in diameter of the cell, and its substance at once contracting after the break to form a ring about the inside of the side wall at the point of junction of the two cells it had formerly separated. This opinion of the early disappearance of cross walls is in agreement with the belief of Hill & Freeman (1903) that young vessels of *Dioscorea* were multinucleate owing to the breakdown of intercellular partitions. Scott (1935) recorded the same early breakdown in primary vessels of *Ricinus*, but in a later, more comprehensive paper (1937) described the multinucleate condition in the coenocytic vessels as due to mitotic division rather than the dissolution of partitions between cells. Moreover, Pirotta & Buscalioni (1898) and Němec (1910) held the same to be true for the Dioscoreaceae.

Esau & Hewitt's paper (1940) was written because earlier findings of the senior author (Esau, 1936*b*) were contrary to those of Priestley, Scott & Malins. Even in forms like *Cucurbita* with exceptionally wide and rapidly growing tracheae, the cross walls were invariably still present after full size had been attained. They consisted, according to these authors, of a pectic layer with very thin layers of cellulose on either side, and in the several species included in the investigation, disappeared only after the secondary thickenings on other parts of the tracheae had reached their full thickness.

It is noteworthy that in much of their work, Priestley, Scott & Malins made use of macerating fluids which dissolve the pectic constituents of cell walls, and even with careful technique, breaking and apparent disappearance of the exceedingly delicate layers of cellulose in the cross walls might well follow after the pectic matrix had in this manner been removed.

*Sieve tubes*

A scrutiny of the literature reveals a number of references to the developmental stages of sieve tubes in various plants. Where companion cells accompany the sieve tubes, the first stage in development is the longitudinal division of the mother cell (Wilhelm, 1880). This, according to Esau's figures (1938*a*), resembles that described by Bailey (1920*b*) as occurring in cambium cells. In many plants with companion cells, these cells are absent from some sieve tubes in the protophloem (Chauveaud, 1900; Chang, 1935; Esau, 1935). In such cases the final longitudinal division of the mother cell may not take place, or it may result in two sieve tubes, or in one sieve tube and a row of bast parenchyma. When the companion cell is present, its nucleus seems from the first to differ from that of the sieve tube in its staining properties and its granular appearance (Lecomte, 1889; Esau, 1934).

The enlargement of the sieve tube vacuole follows, accompanied in many cases by appearance of one or more slime bodies which take various characteristic forms, small drops in Cucurbitaceae (Wilhelm, 1880; Fischer, 1886; Lecomte, 1889; Crafts, 1932), spindle-shaped in Leguminosae (Strasburger, 1891; Baccarini, 1892; Mrazek, 1910; Nelson, 1922; Doolittle & McKinney, 1923; Bailey, 1923*b*), more massive in Solanaceae (Nelson, 1922; Kotila & Coons, 1923; Doolittle & McKinney, 1923; Kofoid, Severin & Swezy, 1923; Artschwager, 1924; Crafts, 1933, 1934; Esau, 1938*a*) and of various shapes in *Vitis* (Wilhelm, 1880) and *Beta* (Esau, 1934). The slime bodies form in the cytoplasm and are of protein. They soon disappear, their substance passing into the vacuole (Fischer, 1885, 1886; Lecomte, 1889).

About this time the nucleus of the sieve tube generally disappears, first swelling and losing much of its ability to absorb stains (Esau, 1938*a*). In a few plants nuclei have been observed in mature sieve tubes (Fischer, 1886; Lecomte, 1889; Schmidt, 1917), but this is exceptional, as shown by the many reports of their disappearance (Wilhelm, 1880; Janczewski, 1881, 1882; Schmidt, 1882; Russow, 1882; Strasburger, 1882, 1887, 1891; Artschwager, 1924; Crafts, 1932, 1933, 1934; Esau, 1934, 1935, 1936*a*, 1938*a*).

In very young sieve tubes, plastids develop in the cytoplasm. They produce a carbohydrate that stains red with iodine (Esau, 1938*a*).

The sieve plate area, when first distinguishable, is merely a thin spot in the cell wall, resembling a simple pit. Callus appears on this first in the form of little plugs opposing each other and extending into the wall from both sides until only a very thin sheet of middle lamella is left between (Russow, 1882; Hill, 1901, 1908). These plugs are formed about fine protoplasmic strands that extend from cell to cell, and at the same time the strands enlarge and change to slime. Hill, who has described this process, assumes an enzymatic action changing protoplasm to slime, enlarging the pores and changing cellulose to callose. He found a number of strands for each callus cylinder in *Pinus* and in some angiosperms, and one for each in others. The amount of callus increases, the cylinders coalescing so that the sieve plate is covered and the whole attains a thickness about equal to that of the cellulose wall between the plates. At this stage, the culmination of the 'active' stage of Janczewski (1881, 1882), the connecting strands are at their thickest. Following this comes Janczewski's 'transitional' stage, when the callus becomes thicker and the strands thin out and finally disappear. The callus then dissolves away, the proto-

plasm of the sieve tube disappears, and the element has reached its 'passive' stage, when its usefulness as a conducting element has ceased.

*Fibres*

The development of flax fibres has been dealt with in two papers by Anderson (1926, 1927*a*). These fibres are part of the primary tissues, but he considered that only part of their length was accounted for by infrequency in formation of transverse walls, the rest being attained by gliding growth. The primary walls, called secondary by Anderson, are of cellulose with a pectic constituent near the middle lamella, and he saw the further thickenings, of pure cellulose, deposited on the outer surface of the cytoplasm, as successive layers of gelatinous consistency, and successively pressed on to the wall. While producing a new layer the cell was partially plasmolysed, and when the layer was complete an increase in turgor pressed and moulded it on to the wall.

The local, bulb-like enlargements which are frequently found, are formed by local stretching of the walls rather late in fibre development, and the protoplasm in the bulbs secretes about itself additional layers of wall. According to Anderson (1927*a*), the thickening layers appear in the lower part of the fibre after the period of rapid elongation. He did not in his paper follow their deposition as it proceeded to the upper end. They are composed of fibrils winding spirally about the fibre, the direction of the spirals being reversed in each successive layer.

Lignification occurs in isolated fibres or fibre groups, and is not uniform over the length of the fibre. Where present, it begins in the middle lamella and extends to the primary wall but not to the secondary.

Bast fibres in *Boehmeria*, where a single one may be 550 mm. in length and extend through several internodes, were studied by Aldaba (1927). No microchemical tests were made, but structurally the development was carefully observed. He found the fibres increasing in length as the plant growing point added new cells to the tissues around them and saw no evidence of gliding growth. The very young fibre was surrounded by a thin membrane the stretching of which readily allowed for increase in length. Soon a second inner membrane formed as an ingrowth from the primary one near the base of the cell, and gradually extended up toward the apex. Later a third began in the same way near the base of the second, and this was repeated a number of times. The layers that had been separated from the cell cytoplasm by subsequently formed membranes thickened and formed the cellulose layers of the mature wall. Elongation in the meantime continued in the upper part of the cell where the secondary layers had not yet reached. When elongation ceased, the layers of the wall continued their upward growth, and as each approached the apex of the cell its rim became attached to the next outer layer. The extra layers in the bulb-like swellings were produced in the same way.

Aldaba found that when the fibres were plasmolysed the inner membrane always remained attached to the protoplasm. In this respect and in most others his description and that of Anderson are in substantial agreement, though often different aspects of the problem were emphasized by the two workers.

In shorter fibres the development apparently follows a different course. It has been studied in *Sansevieria* and *Agave* by Meeuse (1938). Their development begins by longitudinal divisions of a cell to form a group of procambium-like cells, which elongate to 40–60 times the original length. Secondary thickening occurs only when growth in length

has ended, and takes place in all parts of the wall simultaneously. When it is complete, lignification begins. Pectic substances disappear during lignification.

In none of the above researches was any reference made to more than one nucleus in a fibre. Multinucleate fibres are by no means uncommon, however, the condition arising from divisions of the original nucleus without accompanying cytokinesis (Treub, 1880; Kallen, 1882; Saito, 1901; Wilde, 1902; Esau, 1938*b*). In the septate fibre, not only the nucleus, but the cell itself has divided, the cross walls being thin, however, in comparison with the side walls of the fibre.

## Causal morphology

During any developmental study the investigator's mind is constantly assailed by questions of cause. Each new development discovered brings with it the stimulating, often irritating problem of its source. It is not surprising, therefore, that the founder of the science of descriptive developmental morphology should also have initiated investigations on the causal aspect of the subject.

In his *Allgemeine Morphologie der Gewächse* (1868), Hofmeister initiated a new kind of morphology, in which an attempt was made, not merely to describe and compare characteristics of form and structure, but to consider them from the physiological aspect, in an effort to relate them to their proximate causes. The full effect of this attitude on the science of plant morphology cannot yet be estimated. It continues to grow as new discoveries in physics, chemistry, and the physiology of plants provide additional tools for solving the problems that continually present themselves.

Hofmeister's theory of phyllotaxy, that the position of a new leaf primordium was dependent entirely on those of its nearest neighbours, each leaf being situated at the point on the stem circumference farthest from the margins of these neighbours, was opposed by some, as related by Goebel (1926). Later workers, however (Priestley & Scott, 1933; Priestley, Scott & Mattinson, 1937), present views in accord with those of Hofmeister. They hold that the causal factor lies in the fact that each leaf primordium, while meristematic, uses the available nutriment from a segment of the axis limited by the width of its base, and thus prevents the development of a new primordium above it until its meristematic stage is finished. It thus happens that each new primordium is initiated in the widest gap available among its meristematic neighbours.

The development of causal morphology has been retarded by lack of adequate understanding of underlying physical and chemical processes. Papers published from time to time have shown, however, a persistent interest in problems of causation. We have, for example, the controversy between Hartig (1890, 1892) and Jost (1891, 1892*a*, *b*) as to whether cambial growth in spring originates in response to increased food supply, or to an impulse conveyed to the cells of the cambium from the developing leaves, and Kny's demonstration (1889) that for wound periderm formation and suberization in the potato, oxygen is a necessity, while the presence or absence of light has no effect. Numbers of other publications might be listed, which, like the above, deal with causal morphology, but which are not developmental in nature and are outside the scope of the present review.

In later years the interest in causal morphology has been rejuvenated by the work of Priestley and his school. Their method is to follow in as much detail as possible the developmental history of plant structures, morphological, chemical and physical, keeping always in mind the associated problems of cause and effect, and they have built up a

stimulating and challenging structure of hypothesis that is having a notable effect on botanical thought.

The meristematic cells at the apex of one of the higher plants are thought of as having a plastic nature, without osmotically active vacuoles which would cause them to swell greatly. In this way is explained the lack of intercellular spaces (Priestley, 1928) and the cell shape (Tupper-Carey & Priestley, 1924) approaching the tetrakaidekahedron to be expected when plastic spheres are pressed together. The cell walls, according to Tupper-Carey & Priestley (1923), contain cellulose and a soft pectic substance which they speak of as pectin. This pectin is also present in the middle lamella. The typical cellulose reaction with chloriodide of zinc is not given by these walls without pre-treatment with chemicals, while iodine and sulphuric acid will react with the walls of the stem tip but not those of plumule, radicle, or root. The authors believe these peculiarities to be due to masking fatty substance in the cell walls of stem tips, and to fats and protein in roots, and in the radicle and plumule of the seed. It is difficult to prove conclusively that the presence of these particular substances is responsible for the masking effect, but the macrochemical tests suggest their presence closely linked to the cell wall material.

These cells have little tendency to absorb water. It is held (Priestley, 1929) that adjacent vacuolating cells can take water from them. This condition, retained only while the surrounding liquid has a *p*H near the iso-electric point of the main cell protein, is an ideal one for the formation of complex substances from simple ones, with the liberation of water. The cells at this stage are engaged chiefly in the synthesis of more protoplasm, though carbohydrate condensations are not entirely absent, as evidenced by the fact that the cellulose and pectic acid of the thin cell walls are constantly being produced.

The walls are held (Priestley, 1929) to be the channels of transport for food materials from the vascular system to the meristematic cells. When a cell has reached a certain size, the ratio of bulk to area becomes such that the building of more protoplasm is slowed down by the inadequacy of the food supply. Under these conditions cell division takes place, a new cross wall is formed, and conditions for active growth are restored.

The change from non-vacuolated to vacuolated cells is accompanied by an intensification of carbohydrate metabolism. 'Visible vacuoles appear, probably as a result of the release of soluble carbohydrates.' The appearance of intercellular spaces at this stage is held to be due to an increase in elasticity of the cell wall, causing the cells to round up. The constitutional change that provides this elasticity has not been identified.

The change from a slowly vacuolating cell which still divides, to a rapidly extending cell, corresponds in time, according to Priestley's work, with the advent of air into the intercellular spaces. He places these in causal relationship to each other, connecting the phenomenon with Ziegenspeck's (1919, 1920, 1925) amyloid stage, when the cellulose fraction of the wall is said to be very plastic, thus accounting for such phenomena as the growth of root hairs. Some doubt is cast on this theory by later workers, one of whom in a special research on amyloid failed to find this substance in places where Ziegenspeck found it (Hopmann, 1930), while another successfully explained the growth of root hairs by changes in the pectic rather than the cellulose layer of the wall (Cormack, 1935).

The suggested explanations for the fact that the vacuolating cells increase little in thickness, while elongating considerably in a direction parallel to the plant axis, are first the resistance of the cuticle (active in shoot tips but not in roots), and secondly the 'orientation and linkage of cellulose molecules'. The force of the latter explanation is

weakened when one considers that the walls at this stage are assumed by the authors to be of amyloid, not true cellulose. This weakness is intensified by the further suggestion that the protrusion of surrounding cells into air-filled tracheae to form tyloses, a very irregular form of swelling, is brought about by the same causes that lead to the symmetrical elongation we are considering.

The striking difference in organization between shoot and root is tentatively explained on the basis of differences in permeability. The apical meristematic tissues of roots are shown to be much less permeable than those of shoots (Priestley & Tupper-Carey, 1922), a fact explained by the already described permeation of the cell walls of the root tip with protoplasm. In the stem tip water is assumed to pass more easily along the porous walls, carrying food substances that can be absorbed by the cells. As a result, the actively dividing meristematic cells of the shoot extend to the very tip, whereas in the root, the meristematic tissue is nearer the end of the vascular system, with the root cap beyond. Moreover, the root tip has many layers of cells that divide only anticlinally, because they are stretched by the more rapidly dividing cells nearer the source of food supply. In the stem this may or may not be so, for shoot apices whose inner layers divide more rapidly, have a several-layered tunica with anticlinally dividing cells, while the low domes of many gymnosperm shoot tips do not have the outer layers stretched and have little or no tunica (Priestley, 1928).

The exogenous development of leaves and branches is explained on the same basis. At certain intervals, regulated by heredity, the cell division in bits of the outer layers of the stem tip outstrips that of the inner layers, giving rise to folds which become leaf primordia. Such a circumstance does not, according to the theory, happen in the root, because the food supply to the outer layers is more limited, and in roots the branches arise far back from the tip and within the endodermis (Priestley & Pearsall, 1922).

According to the hypothesis of Priestley's school, two conditions must be present in the sap surrounding meristematic cells if they are to continue dividing. There must be a sufficient amount of food, and the *p*H must be at the iso-electric point of the principal proteins of the cell protoplasm. The tentative suggestion is made that the ring of residual meristem (procambium of Priestley, 1928) remains meristematic because the proper *p*H is maintained by a blending of sap from the acid wood and the alkaline bast differentiating just below. This hypothesis leaves some things still to be explained, such as the solid core of residual meristem found in some plants above the protostele.

The procambial cells, failing to vacuolate, and remaining plastic, are pictured as being so pressed between the vacuolating tissues of pith and cortex that they elongate, while remaining thin and narrow. For this reason the discrepancy between surface area through which food is supplied and the volume of protoplasm using this food for synthesis of proteins does not increase greatly with increase in cell size, and so these cells can attain greater bulk before dividing than those of the apical meristem.

When some procambial cells have become wood and others bast, there is a gradient of hydrogen ion concentration in the sap between the two, and the cambium is a layer of cells remaining meristematic at the point in this gradient where the iso-electric point of their principal proteins is reached. The fact that cambial cells divide longitudinally is held to be due, possibly, to this gradient in *p*H, and the authors cite other cases where cell divisions take place similarly at right angles to such a gradient, e.g. the meristematic cells of the root tip, between the acid of the vacuolating cells above and the comparatively

present. They could not be traced to wounds, but always ran through irregularities in the xylem tissue resembling false annual rings, which extended for a much smaller vertical distance than did the canals. Experiment showed that such false rings could be produced in a number of ways—by pinching, compressing or bending a branch, or binding it tightly with string or wire—and the treatment always produced resin canals at the same time. The sensitiveness to the stimulus increased with age and repeated wounding. It was found that every 'normal' canal in such a tree could be traced to such a disturbance, and protected seedlings in sheltered places could grow for six years without a canal.

Thus a new viewpoint was provided for the phylogenist. If all secondary resin canals were due to external stimuli, different intensities of reaction would account for the short canals produced in Abieteae, the longer ones in the spruce, and the still longer and more plentiful ones in the pine. Moreover, the 'normal' canals in cone axes and young branches of mature trees in Taxodineae were explainable by the special strains in these organs, whipped about by the wind, acting on tissues whose sensitiveness had increased as the trees grew older. No theory of conservative organs was necessary, and the argument for the ancestral position of *Pinus* had disappeared.

This series of experiments, having attained its object in so far as the student of comparative anatomy was concerned, was discontinued, and knowledge is still lacking concerning the mechanism through which the stimulus acts to produce the strand of parenchyma and the resin canal. The investigation thus emphasizes an important consideration, namely that researches of an experimental, developmental nature will be more productive if undertaken entirely for their own sake and on material chosen not from any ulterior motive such as its importance in phylogenetic or other theories, or its economic importance. The application of this thesis is illustrated in some recent work.

Cormack (1935) made a study of the development of root hairs, particularly in some species of *Brassica*. The epidermal cells that were to produce root hairs were distinguishable at an early stage, vacuolating later than others and to a lesser extent.

Just before the hairs began to form, a change from pectic acid to calcium pectate occurred in the middle lamella and the corresponding layer of the outer wall of the short epidermal cells on which hairs were produced. The papillae which were the beginnings of hairs were sometimes tipped with pectic acid outside the cellulose and sometimes with calcium pectate, as indicated by solubility tests. The same was true of growing hairs. The amyloid stage, reported by an earlier worker as being present in the cellulose layer (Ziegenspeck, 1920) was not found. Even in hairs with no chemically determined differences between tip and sides, the tip was more delicate and softer than the sides, as shown by the bursting of hairs at the tips when caused to vacuolate rapidly.

Experiments *in vitro* showed that pectic acid when supplied with an amount of calcium insufficient to neutralize it completely, formed a pectate that reacted typically in solubility tests, but was considerably softer than that produced by completely neutralizing the acid.

From the above results the hypothesis was constructed that the hair grew by vacuolation and the continual stretching of the soft tip, the wall of which was constantly added to by the protoplasm as it was thinned by stretching. The pectic acid so added was slowly neutralized by calcium, preventing the sides of the hair from bulging.

Experiments to test this hypothesis invariably strengthened it. Deficiency of calcium ion or the presence of a substance such as an oxalate or a dilute acid, which would hinder calcium pectate formation, stopped the development of hairs, or, when present to a lesser

degree, produced irregularly swollen and branched hairs. An abundance of calcium along with a low hydrogen ion concentration, which would make for very rapid production of calcium pectate, prevented the formation of hairs, and caused such a rapid hardening of cell walls that the cortex was broken and cleft by the continued growth of cells inside the endodermis, where the salts in solution could not penetrate rapidly. When a solution proper for the gradual formation of the pectate was applied, normal growth and hair production ensued.

Experiments with a number of other plants confirmed these conclusions. A plant like *Zea mays*, which fails to develop root hairs in water, was induced to produce them by increasing the alkalinity of the solution so as to neutralize the acid forming in the roots under anaerobic conditions. Incidentally, the long epidermal cells in the *Brassica* roots, which did not produce hairs normally, were found to be more acid than the hair-producing cells, and to have no calcium pectate in their walls at the time of hair production. They were induced to develop hairs when their acidity was neutralized.

In a later paper using similar experimental methods, Cormack (1937) attacked the problem of root hair production by underground roots of *Elodea*, and the lack of such structures in water roots, showing that hairs appeared copiously in water roots deprived of light, and that a root artificially prevented from developing chlorophyll and a cuticle would produce them even in light. His hypothesis was that photosynthesis in the root epidermis set free oxygen necessary for the toughening of a cuticle which would prevent the outgrowth of hairs.

McPherson (1939) became interested in the development of lysigenous air spaces in water plants, and chose the root of *Zea mays* as a suitable material for investigation because its lacunae could readily be modified by experimentally controlled conditions. His observations were not in agreement with those of earlier writers who held that cortical cells were torn apart and killed by the growth of an outer ring of mechanical tissue, for he found that during the period of enlargement the cells which afterward disappeared were always turgid and pressing against each other. The succession of morphological changes was as follows. The cortical cells had the usual beginning of rapid vacuolation, suggested by Priestley to be facilitated by the amyloid condition of the primary wall. McPherson's results agreed with those of Cormack (1935), however, in that he was unable to obtain an amyloid reaction. During the time of the root's increase in diameter the cells were turgid, but those located where air spaces were to form soon afterwards began to collapse. The protoplasm disappeared finally and the walls collapsed entirely, leaving a lacuna.

Experimentally it was found that lacunae formed, but to a lesser extent, with the roots in air, and that their formation could be entirely prevented by an increased concentration of oxygen. After investigation of a series of possibilities the author decided on starvation and autolysis as the probable cause of the disappearance of protoplasm, brought about by the inability of the food transporting mechanism to keep pace with the rapid breakdown of foodstuffs in anaerobic respiration.

A number of other plants were found to act in an identical manner except for apparent respiratory differences, such that each plant had its own particular oxygen concentration below which lacunae were produced, and above which the root cortex remained solid.

The cell walls of the lacuna-forming cortex contained cellulose, and the middle lamella was of pectic acid (not pectin or calcium pectate), as indicated by bright staining with

ruthenium red, comparative insolubility in hot water, and easy solubility in dilute solutions of potassium hydroxide.

Plants whose middle lamella changed promptly to calcium pectate failed to form lacunae though the cell protoplasm died as in *Zea*. The walls failed to collapse. When corn roots were experimentally grown under conditions such that the lamella changed to calcium pectate, they also failed to form spaces.

A later note (Sifton, 1940) has shown that such spaces occurring in some leaves have a similar origin. Here the cells reach an abnormal size before collapsing, and some evidence is shown that the rapid increase is due to the increase in permeability to water on the part of their protoplasm, an increase that apparently continues until the semi-permeability gives way to complete permeability, resulting in collapse.

Watson (1942*a*) was interested in the development of palisade cells, which he studied in the leaves of *Hedera helix* L. Juvenile leaves of this plant have pronounced palisade tissue when grown in strong light, but none at all in shade. When plants with full-grown shade leaves were placed in the sun they slowly developed palisade cells. Almost immediately after the change to sunlight, the sub-epidermal cells died except in proximity to the veins, suggesting desiccation. Stages in the development of palisade in full-grown leaves were carefully studied. The first change after removal to more intense light was an accumulation of starch in the upper layers of mesophyll. Later the starch content of the uppermost cells decreased markedly and this was accompanied by increased osmotic value, and followed by the increased vacuolation that produced the palisade. There was also found a decrease in vacuolar *p*H of these cells from above 6·2 to about 5, accompanied by a lowering in the starch-sugar ratio. The theory that this change came as a result of desiccation was tested by placing comparable leaves, under equality of light and temperature conditions, with their upper surfaces continuously in contact with moist and dry air respectively. Under dry air the decrease in starch content took place, but not under moist. In a comparable experiment using whole plants with roots in nutrient solution, the one in dry air produced palisade while the one in moist air did not. It was found, however, that light of four times the intensity of that used in the above experiments could bring about the development of palisade tissue without the aid of desiccation, a point still to be explained.

The development of the wavy contour in leaf epidermal cells was carefully studied by the same author (Watson, 1942*b*). In *Hedera helix* the epidermal cells of the leaf grown in shade had margins more wavy than those of the sun leaf. The undulations arose rather suddenly during one stage of vacuolation. The margin of the cell always became wavy first where it joined the outer tangential wall, while the lines of junction between radial and inner tangential walls were still straight, and in sun leaves this condition still remained at maturity. Thus it was strongly suggested that the cause of waviness was to be found in the outer free wall. This wall was found to have an inner cellulose layer and an outer cuticle. At the time when the undulations developed, the cuticle stained differentially with Nile blue sulphate. The part over the centre of the cell stained darkly, that over the convolutions lightly. The part that stained lightly was invariably the part that was stretching to produce a convolution. When the cell was mature, differential staining was no longer possible, and stretching had stopped. Watson pointed out that, if the condensation and oxidation processes that are held to take place in cuticle during hardening are considered as extending gradually over the cell surface, cell expansion will be limited at

the points where the hardening first reaches the radial wall, while continued expansion in other places will produce the waviness.

In the group of researches just reviewed the method followed is to choose a well defined and limited structure on the basis of its suitability for intensive developmental and experimental study. This structure, a cell or group of cells that exhibits some particular form or function to an accentuated degree, is then investigated intensively as to its development and its final condition. Next the hypotheses developed in this investigation are subjected to experimental checks and modified if necessary. In this way the investigator not only gains insight into the particular, perhaps very limited and specialized, subject of his investigation, but in doing so uncovers points of general application that have, from lack of such work, hitherto been overlooked. As investigations of this kind increase in number and their results accumulate, the student of developmental morphology may hope to become a contributor, not merely to his own speciality and to morphology in general, but to the other botanical sciences of physiology and ecology, whose interdependence with morphology there has been a tendency in some quarters to overlook.

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# EXPERIMENTS ON COLOUR AND HETEROSTYLY IN THE PRIMROSE, *PRIMULA VULGARIS* HUDS.

By E. M. MARSDEN-JONES and W. B. TURRILL

In the *New Phytologist*, **30**, 284 (1931), we published a short paper on flower mutations in the common primrose, *Primula vulgaris* Huds. (*P. acaulis* L.). About the same time as the experiments recorded in that paper a small amount of research was done in colour factors in British material of the primrose. The results have some interest since they in part confirm and in part extend earlier work. Primroses proved difficult to cultivate at Potterne under controlled conditions. This and the pressure of other research prevented the experiments being carried further.

## Stock plants

| Stock plant (S.P.) no. | Colour, as matched with Ridgway's plates | Style length |
|---|---|---|
| 1 | Tourmaline Pink XXXVIII | Long |
| 2 | Vernonia Purple XXXVIII | Short |
| 3 | Magenta XXVI | Long |
| 4 | Dark Vinaceous XXVII | Long |
| 5 | Daphne Red XXXVIII | Long |
| 6 | Pale Green-Yellow V | Long |
| 7 | Pale Green-Yellow V | Short |
| 12 | (Pale) Seafoam Yellow XXXI | Long |
| 13 | Pale Green-Yellow V | Short |
| 14 | Seafoam Green XXXI | Short |

Stock plants 1–5 came from near Tenby, Pembrokeshire, in 1925, and were communicated by Mr Arnett through the kind offices of the late Mr Miller Christy. Stock plant 6 came from Potterne wood, near Devizes, Wiltshire; stock plant 7 from Eastwell, near Devizes, Wiltshire; stock plant 12 was received from Mr A. D. Cotton, O.B.E., P.L.S., without locality; stock plant 13 came from near Potterne; and stock plant 14 from near Bradfield, Berks.

Most of the breeding work recorded below involved plants from near Tenby and near Potterne. It is, however, interesting to add here that on 30 April 1929, at Paradise Wood, Bradfield, Berks, a population of primroses with some factors for anthocyanin colour was examined. In a narrow strip, 18 × 2 yd. in size and 440 yd. from the nearest house, there were numerous yellow-flowered (Pale Green-Yellow) plants amongst which were twelve with purplish coloured flowers. Of these last eight had flowers Hydrangea Pink on cream ground and four Vinaceous on cream ground.

## Flower colours

The following flower colours, additional to those of stock plants, appeared in bred families or were found in the wild Bradfield population: Hydrangea Pink XXVII, Vinaceous XXVII, Vinaceous Lilac XLIV, Purplish Vinaceous XXXIX, Deep Vinaceous XXVII, Schoenfeld's Purple XXVI, and Indian Lake XXVI. The roman figures in this paragraph

and in the table of stock plants give the plate references to Ridgway's *Color Standards and Nomenclature*.

The plants with purplish coloured (anthocyanin containing) flowers can be arranged in the following sequence, commencing with those with the lightest and ending with those with the deepest colours: Hydrangea Pink, Vinaceous, Vinaceous Lilac, Purplish Vinaceous, Tourmaline Pink, Deep Vinaceous, Daphne Red, Magenta, Dark Vinaceous, Schoenfeld's Purple, Vernonia Purple, and Indian Lake.

## Breeding

### *Selfings*

**M. 14.** S.P. 1 selfed (Tourmaline Pink, long-styled).
9 plants raised to flower: all long-styled; 6 Magenta, 3 Seafoam Yellow.

**M. 17.** S.P. 3 selfed (Magenta, long-styled).
4 plants raised to flower: all long-styled; 2 Magenta, 2 Indian Lake.

**M. 16.** S.P. 4 selfed (Dark Vinaceous, long-styled).
17 plants raised to flower: all long-styled; 2 Pale Green-Yellow, 3 Dark Vinaceous, 12 Deep Vinaceous; both the Dark Vinaceous and the Deep Vinaceous flowers had yellow backgrounds.

**M. 15.** S.P. 2 selfed (Vernonia Purple, short-styled).
No viable seed set.

**M. 18.** S.P. 5 selfed (Daphne Red, long-styled).
No viable seed set.

### *Crossings*

**M. 12.** S.P. 13 (Pale Green-Yellow, short-styled) × S.P. 5 (Daphne Red, long-styled).
31 plants raised to flower:
10 Pale Green-Yellow, of which 7 long-styled, 3 short-styled.
12 Vernonia Purple, of which 8 long-styled, 4 short-styled.
8 Deep Vinaceous, of which 6 long-styled, 2 short-styled.
1 Vinaceous Lilac, of which 0 long-styled, 1 short-styled.
In the last two colour classes there was an obvious yellow background to the dominant colour.

**M. 13.** S.P. 13 (Pale Green-Yellow, short-styled) × S.P. 1 (Tourmaline Pink, long-styled).
34 plants raised to flower:
20 Pale Green-Yellow, of which 9 long-styled, 11 short-styled.
9 Deep Vinaceous, of which 5 long-styled, 4 short-styled.
5 Hydrangea Pink, of which 2 long-styled, 3 short-styled.
In the Deep Vinaceous class a yellow background could be distinguished.

**M. 19.** S.P. 7 (Pale Green-Yellow, short-styled, with enations) × S.P. 1 (Tourmaline Pink, long-styled).
No viable seed set.

**M. 20.** S.P. 1 (Tourmaline Pink, long-styled) × S.P. 2 (Vernonia Purple, short-styled).
45 plants raised to flower:
10 Pale Green-Yellow, of which 4 long-styled, 6 short-styled.
1 Seafoam Yellow, of which 1 long-styled, 0 short-styled.
16 Schoenfeld's Purple, of which 15 long-styled, 1 short-styled.
9 Vernonia Purple, of which 7 long-styled, 2 short-styled.
6 Daphne Pink, of which 4 long-styled, 2 short-styled.
3 Deep Vinaceous, of which 2 long-styled, 1 short-styled.

**M. 24.** S.P. 12 (Pale Seafoam Yellow, long-styled) × S.P. 7 (Pale Green-Yellow, short-styled, with enations).
13 plants raised to flower: all Pale Green-Yellow, with no enations; 6 long-styled, 7 short-styled.

**M. 25.** S.P. 14 (Seafoam Green, short-styled) × S.P. 7 (Pale Green-Yellow, short-styled).
No viable seed set.

**M. 26.** S.P. 1 (Tourmaline Pink, long-styled) × S.P. 7 (Pale Green-Yellow, short-styled, with enations).
10 plants raised to flower, none with enations:
3 Purplish Vinaceous, of which 2 long-styled, 1 short-styled.
7 Pale Green-Yellow, of which 3 long-styled, 4 short-styled.
Owing to weathering of the flowers the scoring of one of the Purplish Vinaceous plants was doubtful.

**M. 27.** S.P. 5 (Daphne Red, long-styled) × S.P. 7 (Pale Green-Yellow, short-styled, with enations).
32 plants raised to flower:
22 Pale Green-Yellow, of which 7 long-styled, 15 short-styled.
10 Purplish coloured, of which 4 long-styled, 6 short-styled.
None with enations. Of the plants with purplish coloured flowers, 4 had Dark Vinaceous, 1 Vernonia Purple, 1 Purplish Vinaceous, and the remainder were not exactly scorable owing to the weathered condition of the flowers.

## DISCUSSION

*Enations.* In the crosses M. 24, M. 26 and M. 27, the pollen parent (S.P. 7) had petal enations, while the ovule parent (S.P. 12, S.P. 1 or S.P. 5 respectively) had none. All the $F_1$ offspring (a total of 55 plants) were without enations. This agrees with our earlier published results which indicated that the presence of enations was recessive to their absence.

*Heterostyly.* The effective selfings were all of long-styled plants and all bred true to the long-style character. This is according to expectation, since the long-styled character has been shown to be normally recessive in the common primrose.

All the short-styled stock plants (S.P. 2, S.P. 7 and S.P. 13) used in crosses were heterozygous for this character, since when they were crossed with a long-styled plant (whichever way the cross was made) there was segregation for 'pin' and 'thrum' flowers. The expected 1:1 ratio was exactly or very closely reached in M. 13, M. 24 and M. 26, but

other families showed considerable divergencies, in two long-styled plants and in one short-styled plants being present in unexpectedly large numbers relative to the respective allelomorph. The summated $F_1$ totals are: 92 long-styled, 73 short-styled. The divergencies from expected ratios are no greater than we have found in earlier experiments or than have been recorded for other more or less comparable controlled breeding with species of *Primula* by other authors. They are probably due to a variety of causes, including low numbers and reduced viability of certain genic combinations, especially those with expected 'thrum' structure of the flowers.

*Flower Colour.* Chittenden (*J. Genet.* **19**, 285, 1928) investigated flower colour in crosses between 'yellow' *Primula vulgaris* (*P. acaulis*) and *P. juliae*, and concluded that 'it is probable that *Juliae* contributes two factors affecting anthocyanin colour in the flower, a colour factor **R** and an intensifier **D**. In addition a factor **Y** for yellow is contributed by *acaulis*'. Buxton published an account of "The genetics of the Wisley blue primrose' (in *J. R. Hort. Soc.* **51**, 305, 1926) and another on the 'Genetics of the primrose *P. acaulis*' (in *J. Genet.* **25**, 195, 1932). Buxton's papers have considerable bearing on our results.

It would appear that Buxton had considerable difficulty in scoring the colours of his primroses against standards. We used Ridgway's *Color Standards and Nomenclature*, but a complication has arisen in that some of the colours shown in Buxton's plate VI (loc. cit.) would not be scored by us into the Ridgway colours whose names he uses.

All our plants with anthocyanin used as stock plants (S.P. 1–5) came from the same (Pembrokeshire) population and 1, 3 and 4 certainly, and probably 2 and 5 also, were heterozygous for colour factors. 'Pale Green-Yellow' is the nearest Ridgway match for the ordinary 'yellow' primrose of the Potterne district. All our selfings and crosses involving anthocyanin flowers involved none other than the stock-plants 1–5 and 'Pale Green-Yellow' flowered plants.

Our results, like those of Buxton's experiments, though from somewhat different angles, suggest that the flavones of the yellow and the anthocyanins of the other primroses are formed independently of one another. In all the $F_1$ families, whether one parent had 'Pale Green-Yellow' and the other anthocyanin flowers or both had anthocyanin flowers, there was segregation with some 'Pale Green-Yellow' offspring. The largest family from the selfings of anthocyanin-flowered stock plants (M. 16) also showed segregation of 'Pale Green-Yellow' flowered offspring.

It is obvious from the results of the selfing M. 14, combined with those from the crossings M. 13, M. 20, M. 24 and M. 26, that 'Pale Green-Yellow' is dominant over the paler 'Seafoam Yellow' (or 'Pale Seafoam Yellow'), or the former is homozygous and the latter heterozygous, for a dominant factor. The results of all the experiments are explicable, so far as 'yellow' (flavone) and anthocyanin are concerned, if yellow flavone was present in all our stock plants with anthocyanin coloured flowers, and there is good evidence for this, and all the plants were heterozygous for an anthocyanin-producing factor or factors, due allowance being sometimes made for the smallness of the families in considering the actual figure values of the ratios obtained.

This leaves the problem of factor or factors for anthocyanin production. Buxton in his 1926 paper accepts the following factors for flower colour in the primrose: **Y** for yellow flowers, **B** for the anthocyanins, **N** an intensifier of the anthocyanins, affecting shade but not colour, and **S** (or **D**) for selective permeability of the cell membrane. In

his 1932 paper he introduces another factor **W** for 'ivory white'. The factor **Y** is acceptable and **W** does not enter into our experiments. We did not obtain any 'blue' flowered plants in any of our experiments, so far as can be judged from the 'blue' flower figured by Buxton (loc. cit. plate VI). He does not appear to give a colour chart name to this 'blue' in the text of either of his papers. Our series of stock plants and offspring from selfings and crosses differ from those in Buxton's 1932 paper not only in the absence of **W** but also in the presence of **Y**, presumably in all the stock plants and in at least the vast majority of the offspring. This constitution probably accounts for the absence of 'blues' in our cultures, the plural being used here since Buxton's 'blue' primroses were not all the same in colour expression. On the other hand, Buxton, several times, obtained lower figures for his 'blues' than were expected and our numbers are small compared to many of his.

The factor **B**, for anthocyanin production, is doubtlessly the same in our material as in Buxton's. The **S** or reddening factor may be accepted as present, in single or double dose, in our stock plants with anthocyanin coloured flowers. As Buxton suggests, it and its allelomorph may act through selective permeability, in the reds (**SS** or **Ss**) the K-ions (or other strongly basic ions) being excluded from the cell and in the blues (**ss**) being admitted with corresponding changes in the *p*H of the cell sap and in colour expression. The **N** (intensifier) factor, or some approximate equivalent, would also appear to be present in some of our plants giving deeper and, on the whole, duller colours.

It is very probable that both the Pembrokeshire and Berkshire populations of anthocyanin primroses owe their sap pigment to genes introduced from gardens. They might provide interesting material for studying the spread of such genes through wild populations. 'Red' primroses are common in the Nearer East and are generally recorded under the varietal names of *rubra* Sibth, et Sm. or *rosea* Boiss. In some areas, as in parts of eastern Bulgaria, they occur to the exclusion of the yellow primrose. It would probably throw further light on the constitutions and origins of the anthocyanin primroses of gardens if wild stocks from such a locality could be genetically analysed.

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# A CONTRIBUTION TO THE EMBRYOLOGY OF *RUDBECKIA BICOLOR* NUTT

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(With 9 figures in the text)

## Introduction

From some material collected near Urbana, Illinois, Palm (1934) published a preliminary note describing a new type of embryo sac in *Rudbeckia hirta* L. The nucellus has a single megaspore mother cell. Reduction division results in four megaspore nuclei which are, however, not separated by cell walls. One nucleus lies at each pole of the cell and two in the middle. The micropylar nucleus continues to remain in its original position but the two lateral ones move to the bottom. Two of these three megaspore nuclei now become cut off at the chalazal end by means of membranes to give rise directly to antipodal cells. The third takes up a more central position and gradually becomes larger. The micropylar megaspore nucleus is said to undergo two divisions to give rise to the quartet forming the egg apparatus and the upper polar nucleus. At the time of its second division the third megaspore nucleus lying near the centre also divides; one of the daughter nuclei becomes surrounded by a membrane to form the third antipodal cell while the other functions as the lower polar nucleus. This last formed antipodal is the largest of the three and the lower polar nucleus is also larger than the upper.

The whole interpretation seems to be rather ingenious and a few years ago one of us (Maheshwari, 1937) called attention to the similarities in embryo sac development between *Rudbeckia* and *Fritillaria*. Further comment was withheld in the hope that Palm's fuller paper would soon be available. Since this expectation has not been fulfilled and a decade has passed since the publication of Palm's preliminary note, we thought it might be useful to go into the question in greater detail.

## Materials and methods

The only species of *Rudbeckia* available to us was *R. bicolor* Nutt, seeds of which were obtained from a nurseryman and grown in the Dacca University Botanical Garden. Fixations were made in the summer of 1942 in formalin-acetic-alcohol and again in 1943 in both formalin-acetic-alcohol and Nawaschin's fluid. With the latter a short pretreatment with acetic alcohol (1 part acetic acid: 3 parts alcohol) improved the fixation. The materials were stored in bulk in 70 % alcohol and then flowers of desired stages were separated out and imbedded in paraffin in the usual way. In the case of older ovaries containing seeds a very brief pretreatment with dilute hydrofluoric acid facilitated cutting and was found to cause no appreciable damage to the internal structures. Sections were cut at a thickness of 7–15 microns and stained in iron-haematoxylin and safranin-fast green.

## ORGANOGENY OF THE FLOWER

The flower primordium arises as a dome-shaped structure in the axil of a bracteole. The petals differentiate first, then the stamens and last of all the carpels. No sign of sepals is seen even in later stages.

### *Microsporogenesis and male gametophyte*

The earliest stages in our material showed the primary parietal and primary sporogenous layers already formed. The former divides periclinally to form two layers of cells of which the outer divides again, resulting in a total of three wall layers.

The innermost layer becomes the tapetum. Its cells are uninucleate at first but later the nuclei divide mitotically and the daughter nuclei fuse together and divide again. The formation of such polyploid nuclei has also been met with in many other members of the Compositae, e.g. *Lactuca*, *Leontodon*, *Anthemis*, *Chrysanthemum*, *Artemisia*, *Achillea*, *Erigeron* (Poddubnaja-Arnoldi, 1931) and others.

There are usually one to two primary sporogenous cells in each anther lobe as seen in a transverse section. These divide to give rise to the pollen mother cells of which three to five are seen in each lobe in a transverse section. The pollen mother cells undergo the usual reduction divisions resulting in tetrahedral tetrads. At this stage, the walls of the tapetal cells disintegrate and their contents float in the anther loculus forming a periplasmodium.

The first division of the microspore usually takes place at the secondary four-nucleate stage[1] of the embryo sac. The spindle is organized towards the wall as a result of which a small generative cell and a large tube cell are formed. The former divides again to form the male gametes, so that the mature pollen grain is three-celled at the time of shedding. This is the usual condition in the Compositae. Only Banerjee (1940*a*) reported two-nucleate pollen in *Tridax procumbens* and (1940*b*) uninucleate pollen in *Carthamus tinctorius*, but Raghavan & Venkatasubban (1941) have shown that the pollen grains of *Tridax procumbens* are three-celled and we have found the same in *Carthamus tinctorius*.

### *The ovary and the ovule*

The ovary is unilocular and inferior. The ovule arises as an erect protuberance from the floor of the ovary, but due to unilateral growth it finally assumes a position of complete anatropy. There is a single integument which becomes apparent even during early stages. The nucellus consists of a single layer of cells. Degeneration of the nucellar cells begins at the primary four-nucleate embryo sac stage or slightly earlier and is almost complete before the secondary four-nucleate embryo sac stage is reached. The nucellar cells at the micropylar portion are the last to disappear.

The degeneration of the nucellar cells is accompanied by the differentiation of an integumentary tapetum from the innermost layer of the integument. During earlier stages its cells are radially elongated but at about the time of fertilization and after that, the cells become stretched so that their longest dimension is parallel to the long axis of the embryo sac. In a longitudinal section of the ovule at the primary four-nucleate embryo sac stage the integumentary tapetum is seen to be about ten to twelve cells high

[1] As will be seen later, the embryo sac of *Rudbeckia* has, like *Fritillaria*, two four-nucleate stages, the first of which is called the primary and the second the secondary four-nucleate stage.

but at the eight-nucleate stage the number increases to about fourteen and in post-fertilization stages to even eighteen or twenty. The cells remain uni-nucleate throughout.

### *Megasporogenesis*

As is customary in the Compositae no parietal cell[1] is cut off and the hypodermal archesporial cell functions directly as the megaspore mother cell (Fig. 1) which undergoes a great increase in size. No walls are laid down between the daughter nuclei following the first or second reduction division (Figs. 2 and 3). In the second division the spindles are placed at right angles to each other, so that when the micropylar spindle shows the polar view the chalazal is seen in the side view. Four nuclei are now formed which are arranged along the length of the mother cell (Fig. 3). No secondary spindle fibres were observed.

### *Embryo sac*

As there is no wall formation during the reduction divisions and no degeneration of any megaspore nucleus, the embryo sac is tetrasporic. In this, i.e. the primary four-nucleate embryo sac, two conspicuous vacuoles arise, one at each end of the embryo sac (Fig. 3). In later stages the rest of the cytoplasm also begins to show vacuolation and soon the denser cytoplasm becomes restricted to the positions around the nuclei and along the embryo sac membrane (Fig. 4).

Of the four nuclei the two centrally placed ones move towards the base of the embryo sac and together with the chalazal nucleus they become arranged in the form of an inverted triangle ▽ (Fig. 4). This 1 + 3 arrangement of the megaspore nuclei is also seen in Palm's Fig. 5.

The next stage in our preparations showed two mitotic spindles of which the chalazal is much broader than the micropylar. The vacuoles at the two ends still persist and in addition a large vacuole has arisen in the centre. Our Figs. 5 and 6 showing this stage are essentially similar to Bambacioni's (1928) Figs. 15 and 17, which leaves no doubt that the development is identical in both cases. Unfortunately, we were not able to see an actual fusion of the three spindles in the chalazal region but this is largely a matter of chance.

At the end of this division we once again get a four-nucleate stage with a pair of nuclei at each end, of which the lower two are distinctly larger than the upper and also contain more than one nucleolus each, indicating their polyploid nature (Fig. 7). This is therefore the secondary four-nucleate embryo sac stage corresponding to Bambacioni's (1928) Fig. 18 in which the two micropylar nuclei are haploid and the two chalazal nuclei are triploid. There is no mention of such a stage in Palm's (1934) paper, who envisages an increase in size of one of the three chalazal nuclei. In our opinion it is at this point that he went wrong and became prejudiced in the interpretation of the subsequent stages.

The four nuclei now divide to form eight of which those belonging to the chalazal quartet are larger than those of the micropylar (note the disparity in size of the upper and lower polar nuclei in Fig. 8).

Degeneration of the nuclei or cells at the micropylar end was frequently met with. Fig. 8 represents one such case in which the antipodals and the polar nuclei are quite

[1] Datta (1939) reported that on account of the division of the 'cover cells' the megaspores become deep-seated in *Mikania cordifolia*, *Launaea asplenifolia* and *Blumea laciniata*. Venkateswarlu (1939) has, however, denied the existence of any 'cover cells' in *Launaea* and it is very likely that Datta's sections were not median.

healthy but the egg apparatus presents a degenerated appearance. This is rather unusual as in most plants the antipodals degenerate first. One is tempted to infer that the polyploid nature of the latter in *Rudbeckia* confers upon them a greater power of resistance.

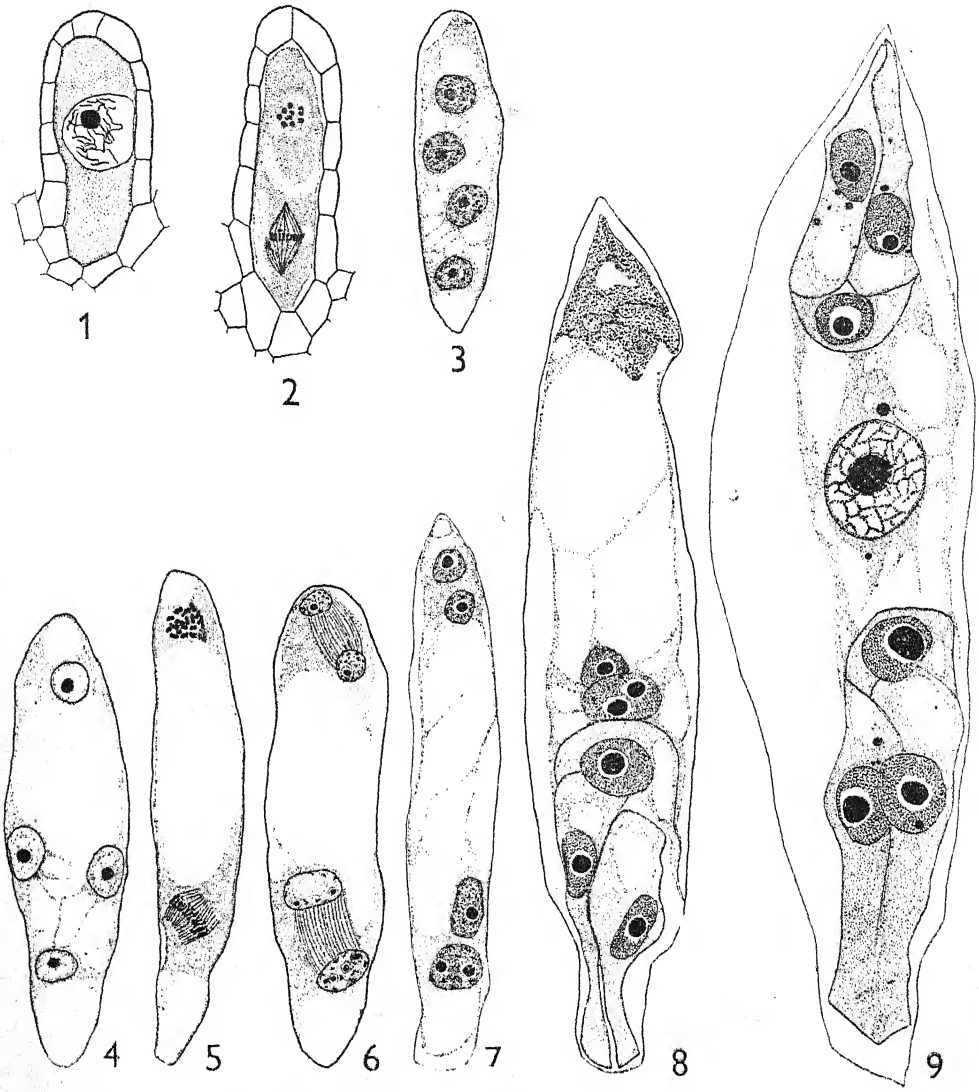


Fig. 1. Megaspore mother cell surrounded by a single layer of nucellar cells. All figs. ×605.
Fig. 2. Second division of the megaspore mother cell. Note the positions of the spindles which are at right angles to each other.
Fig. 3. Primary four-nucleate embryo sac.
Fig. 4. Same, older stage showing 1+3 arrangement of the megaspore nuclei.
Fig. 5. Metaphase stage of the next division. Note the larger size of the chalazal spindle indicating its composite origin.
Fig. 6. Telophase of the above division. Note the disparity in size between the micropylar and chalazal nuclei. The chalazal nuclei have more than one nucleolus each.
Fig. 7. The secondary four-nucleate embryo sac.
Fig. 8. Eight-nucleate embryo sac with the egg apparatus in a degenerating condition. Note the larger size of the lower polar nucleus and the resemblance of the antipodals to an egg apparatus.
Fig. 9. Mature embryo sac. The polar nuclei have fused to form the secondary nucleus.

Fig. 9 shows the mature embryo sac stage. The synergids are pear-shaped and possess definite hooks. As usual the nucleus is situated above the basal vacuole. The egg is in the centre, projecting out below the synergids. The nuclei of the egg and the synergids are of almost the same size. The embryo sac itself is rather large, widest in the middle and narrowing at the two ends.

In size as well as in relative position the group of antipodal cells shows a remarkable resemblance to an egg apparatus. The two lateral antipodal cells, with their nuclei below the basal vacuoles, resemble the synergids and the central antipodal with its nucleus and main mass of cytoplasm towards the broadened upper end, looks like an egg cell. Indeed, looking at the embryo sac alone, one is quite likely to be misled in deciding which is the micropylar and which is the chalazal end. The two lateral antipodal cells (like the synergids) degenerate soon after fertilization but the large central one persists and can be distinguished even up to the time of differentiation of the cotyledons.

Some dark bodies, looking like small nucleoli, are found scattered in the mature embryo sac; a particularly large one may be seen just above the secondary nucleus in Fig. 9. It was first mistaken for the nucleolus of the male gamete but on closer study this appeared improbable since it has no nuclear membrane around it and similar bodies of all sizes are found even in the synergids and antipodals (Fig. 9). It is possible that they may be fragments of nucleoli extruded during nuclear divisions but we have been unable to decide this point. Palm's (1914*b*) Fig. 1*a* shows similar bodies in the embryo sac of *Tanecetum vulgare*.

### *Endosperm and embryo*

We were not able to follow the sequence of divisions in the endosperm and the youngest stage in our material already showed a cellular endosperm. Whether this is preceded by a free nuclear stage or the endosperm is cellular from the very beginning, we are unable to say. Palm (1934) reports the latter to be the case in *Rudbeckia hirta*.

In embryo development the earliest stage that we obtained was one in which the dermatogen has become differentiated by oblique wall formation in the octant cells. As early as this stage, the hypodermal layer of the pericarp shows the deposition of some hardening material.

Further divisions in the embryo result in a globular mass of cells in which the cotyledons now begin to differentiate. The suspensor is uniseriate and six-celled at this stage. The large egg-like antipodal cell which lies imbedded in the endosperm now degenerates. It was never seen to contain more than one nucleus.

The mature embryo is of the usual type seen in other Compositae. In the seed the whole of the endosperm except one or two layers of cells has been consumed. Of the integument also only two or three layers remain and none of these shows any thickenings. Protection for the seed is thus afforded almost entirely by the pericarp in which the hypodermal layer is specially thickened although the epidermis is also cutinized. The other layers of the ovary wall degenerate and are not seen in the mature fruit.

## Discussion

### (*a*) *Palm's observations*

Palm (1934) noted that the development of the embryo sac in *Rudbeckia hirta* did not fit in with any of the existing types. From his Figures 1–11 it is evident that the embryo

sac is tetrasporic and ultimately eight-nucleate. He seems to have been unaware of Bambacioni's (1928) paper on *Fritillaria* and does not take into consideration the possibility of a fusion of the chalazal nuclei or their spindles following the two meiotic divisions, but supposes that, (i) following the 1+3 arrangement there is an increase in size of one of the megaspore nuclei at the chalazal end, and that (ii) only this enlarged megaspore nucleus divides again while the other two directly give rise to two antipodal cells. Thus, while there are two divisions of the micropylar megaspore nucleus, there is only one division of just one of the three nuclei in the chalazal part of the embryo sac.

### (*b*) *Our results*

Our observations on *Rudbeckia bicolor* clearly show that:

(i) All the megaspore nuclei are equal in size and remain so. But following the 1+3 arrangement of the nuclei there is a composite division of the three chalazal ones. There is no increase in size of any individual nucleus at this stage or afterwards.

(ii) Due to the fusion of the spindles at the chalazal end, the primary four-nucleate stage is followed by a secondary four-nucleate one. Being triploid, the two chalazal nuclei are now considerably larger than the micropylar which are haploid. It is evident that Palm has completely missed this stage.

(iii) We never saw a five-nucleate embryo sac as shown in Palm's Fig. 6. The only way to interpret this figure is by assuming an extremely precocious division of the micropylar megaspore nucleus. In our preparations no such precocity was seen but we encountered one case of an opposite nature where the micropylar nucleus was in a resting stage while there is a compound spindle at the chalazal end. It is not impossible, however, that the reverse may also happen under certain conditions (vide Puri, 1939, Fig. 30).

(iv) All the three antipodal cells and the lower polar nucleus are the result of division of the two triploid nuclei of the secondary four-nucleate embryo sac. There are no undivided megaspores directly giving rise to the antipodals as supposed by Palm.

It is interesting to note that another botanist (Hrubý, 1934) made a similar misinterpretation in the case of *Erythronium dens canis*. He got a 1+3 arrangement of the megaspore nuclei beyond which the development was not traced. He nevertheless conjectures that the nucleus on the micropylar end will divide twice and of the chalazal nuclei one will divide once and the other two will remain undivided.[1]

### (*c*) *The 'antipodal oosphere' in the Compositae*

In *Rudbeckia* there are three antipodal cells which as mentioned before show a perfect resemblance to an egg apparatus. The large central antipodal cell has its nucleus above and a vacuole below and consequently looks very similar to an egg cell. It is seen to persist even in post-fertilization stages and appears like an undivided egg. We did not come across any case where it actually functioned as a zygote but as we have only a few preparations of post-fertilization stages, the possibility that it may sometimes give rise to an embryo and some triploid races of plants is not ruled out.

[1] In a later paper, Hrubý (1938) reports that all the four megaspore nuclei divide simultaneously, one mitotic figure being present at the micropylar end and three at the chalazal end. The six nuclei at the chalazal end often approach each other very closely and then begin to degenerate although not with the same speed. The two micropylar nuclei divide normally to form the egg apparatus and one polar nucleus. One nucleus from the chalazal end moves up as the lower polar. The number of nuclei at the chalazal end is variable due to degeneration. In *Erythronium helenae* and *E. tuolumnense*, Cave (1942) has reported the *Fritillaria* type and if Hrubý's (1938) observations are correct, the genus *Erythronium* shows a transition between the *Drusa* type and the *Fritillaria* type of development.

An 'antipodal oosphere' was first reported in the Compositae by Chamberlain (1895) in *Aster Novae-Angliae* which was later confirmed by Goldflus (1898) and Opperman (1904). It is not clear, however, whether this cell really functions as an egg in *Aster Novae-Angliae*, but in *Aster undulatus*, Opperman (1904) reported its fertilization by a male nucleus.

There is also some difference of opinion regarding the origin of this antipodal oosphere in *Aster Novae-Angliae*. While Palm (1914*a*) thinks that it is formed by a persistent megaspore, Chamberlain (1918) reiterates his previous view that it is of antipodal origin. Carano (1921) on the other hand seems to have interpreted it as an enlarged cell of the integument.

There are, however, other undoubted cases where in organization as well as behaviour the antipodal cells show a close resemblance to an egg-apparatus. Tretjakow (1895) first saw this in *Allium odorum* which was later confirmed by Haberlandt (1922) and Modilewski (1925, 1931). The latter says that of the three antipodals two develop and later degenerate entirely like the synergids while the third remains active and even gives rise to an embryo. Shattuck (1905) also occasionally saw in *Ulmus americana* an egg cell in the antipodal region which grows into an embryo.

Chiarugi (1925, p. 232) describes a striking antipodal apparatus in *Cistus laurifolius*, in which two antipodals are similar to synergids and the third is identical with an oosphere. Other instances of antipodal eggs have been reported in *Ulmus hollandica belgica* (Leliveld, 1935), *Nothoscordum fragrans* (Stenar, 1932) and *Allium paradoxicum* (Weber, 1929, p. 33). The case of *Rudbeckia* is similar,[1] although, as previously pointed out, we have up to this time failed to observe any antipodal embryo. This is, however, not impossible or even unlikely, for the antipodal cells are not haploid but triploid and may not require the stimulus of fertilization in order to develop further.

### (*d*) *Other tetrasporic embryo sacs in Compositae*

Tetrasporic embryo sacs are confined to only a few genera in the Compositae, viz. *Erigeron*, *Pyrethrum* (= *Chrysanthemum*) and *Tanacetum*. In all of these the development is a modification of the *Peperomia* type (Maheshwari, 1937). Only in *Pyrethrum balsaminatum*, Ward (1880) reported the *Lilium* type (= *Adoxa* type) but this needs confirmation. In *Leontodon hispidus*, Bergman (1935) reports that the development is usually of the normal type but *Adoxa* type also occurs. The *Fritillaria* type was so far unknown in the family and *Rudbeckia bicolor* is the first case of this kind. There is no doubt that Palm's species *R. hirta* is also similar.

## Summary

1. The floral organs arise in the following succession: petals, stamens and carpels. The sepals are not formed.

2. The anther has three wall layers: the endothecium, the middle layer and the tapetum. The tapetal cells become binucleate but the two nuclei fuse and subsequently divide again, thus giving rise to giant polyploid nuclei. A periplasmodium is formed after the microspore tetrad stage. The pollen grains are trinucleate at the time of shedding.

3. The embryo sac is tetrasporic and eight-nucleate and the development is of the *Fritillaria* type.

[1] In the Compositae antipodal cells of the type seen in *Rudbeckia* are of rather unusual occurrence. Dahlgren (1924, p. 184) reports a giant antipodal cell ('Riesenantipode') in *Ursinea anthemoides* and on two occasions in *Arctotis stoechadifolia* he saw that one of the antipodal cells had enlarged and assumed an egg-like appearance.

4. One of the antipodal cells resembles an egg cell and is suggestive of an 'antipodal oosphere' such as is known in some other Compositae. This persists for a long time and could be recognized even up to the time of differentiation of the cotyledons.

5. Palm's (1934) observations on *Rudbeckia hirta* have been reinterpreted in the light of our results and it is concluded that the *Fritillaria* type occurs in *R. hirta* also.

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# SOME ASPECTS OF TAXONOMY IN THE CRYPTOPHYCEAE

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CONTENTS



## I. Introduction

The class Cryptophyceae is one of the best defined among Flagellata. Although there is possibly some relation to Dinophyceae, the lines of demarcation are perfectly clear. Furthermore, there seems to be no transition to a true algal state, with the possible exception of *Tetragonidium* (cf. p. 144).

Characteristic features of Cryptophyceae are: the peculiar, asymmetrical shape of the cell; the two slightly different flagella, exhibiting a special kind of movement and causing a characteristic type of locomotion, generally recognizable under low power, and involving a change of direction at the slightest irritation; in addition, there is a jerky backward movement caused by stronger harmful influences; the single contractile vacuole near the front end; the inability to form a cell wall; the absence of sexual reproduction; the possession of trichocysts lining a furrow or a gullet; the one or two parietal chromatophores and the deposition of starch as a reserve material.

Diversity within the class comprises: absence or presence of a gullet; size and shape of the cell; the kind of accessory pigments, apart from chlorophyll, and their concentration, with the resulting variation in the tint of the chromatophores; possession or lack of pyrenoids; and in rare cases the existence of an eye spot. In a few species chromatophores are lacking.

The uniformity of organization renders a subdivision of the class into orders, families and genera difficult, the more so as only few species have been adequately described. The reasons for the lack of solid knowledge are: the scarce occurrence of many Cryptophyceae, which are often found in relatively small numbers amid a rich flagellate population; the complicated shape of the body which is not readily represented by drawings; the abundance of starch which often obscures the internal structure of the cell; the incessant mobility and difficulty of fixing the organisms for closer examination and for staining.

Some of these handicaps can be overcome by cultivation, whereby many uniform cells, all originating from one individual, are obtained. Such material can be examined

and re-examined at leisure, and newly discovered forms can be compared with others previously isolated and brought into culture.

Many workers on algae are still inclined to look with suspicion on the use of algal cultures for morphological and taxonomic purposes. This was to a certain degree justified in Chodat's days, but since then the methods have been improved as I shall show in another publication. Culture material is now often healthier and shows the characteristic features more clearly than that found in nature where collections may be made too late to catch the organism at its period of optimal growth. Provision must of course be made to ensure that diagnoses, based on culture material, are so wide as to cover also the modifications which are liable to be found in nature.

I am collecting strains of Cryptophyceae, with the object of preparing a synopsis of the class to facilitate identification which at present is almost impossible. It is no mere accident that so good an observer as Lund (1942, pp. 69 et seq.) merely designates the forms he describes as A, B and C. Although my accumulated experience as to specific features among Cryptophyceae is still very incomplete, certain general conclusions concerning the taxonomy of the class can already be drawn with the help of the relevant literature.

## II. TAXONOMIC FEATURES

In contrast to the ease with which Cryptophyceae can be distinguished from other Flagellata, no adequate basis for classification within the class has so far been proposed. Pascher (1913), so far as I know, has alone made an attempt in this direction. New observations made since that date necessitate a re-examination and partial abandonment of his proposals. One of the first questions is: which features are suitable for establishing natural groups?

### (1) *Non-motile stages*

I cannot support Pascher's (1911*b*, 1912*b*, 1913, p. 100) taxonomic distinction between forms which are motile for the greater part of their lives and those which are usually found in a palmelloid state and form swarmers only occasionally. In my cultures almost every strain will assume the palmelloid state under certain conditions, although the tendency is more strongly marked in some strains than in others.

Pascher's Phaeocapsaceae, embracing those forms which usually occur as palmellae, comprise *Phaeococcus* and *Phaeoplax*. *Phaeococcus Clementi* (Men.) Borzi and *P. adnatus* (Naeg.) West are probably Chrysophyceae (cf. also Fritsch, 1935, p. 661).[1] *Phaeoplax marina* (Reinisch) Pascher appears not to differ in any way from those species of *Cryptomonas* which commonly form palmellae and can, indeed, remain for an indefinite period in this state.

The only member of Cryptophyceae which is described as actually coccoid, and therefore truly algal, is *Tetragonidium verrucosum* Pascher. It was only once found by this author (Pascher, 1914, p. 160), very shortly described and not figured. The description does not make it clear what has actually been observed. Figures drawn by Pascher have been reproduced by Fritsch (1935, p. 660), and these, though instructive, are not altogether convincing because the transitions between the algal and the flagellate stages are not shown.

[1] Oltmanns (1904, p. 14) was of the same opinion; but in the second edition (1922) he yields to Pascher's influence.

(2) *Cell shape*

A better subdivision can be based on the presence or absence of a gullet, a feature distinguishing Pascher's Cryptomonadeae and Cryptochrysideae. The latter are provided only with a furrow, in which the trichocysts are situated. Pascher regards them as more primitive than those Cryptophyceae in which trichocysts are located in a more or less deep excavation. It is not altogether certain that trichocysts occur in every member of the class.

Cryptochrysideae, embracing *Cryptochrysis*, *Chroomonas*, *Rhodomonas* and *Hillea*, with one exception,[1] have hitherto been recorded and described only by Pascher, although 30 years have elapsed since his last publication on the matter. This may be due to the fact that it is not easy to differentiate between trichocysts situated in a gullet or furrow, but if this is so, this feature is not very useful for hydrobiological work, even if the difference is actually valid.

Under such circumstances it is not surprising that there are controversies as to the grouping of certain forms. For example, *Chroomonas Norstedtii*, placed among Cryptochrysideae by Pascher (1912*b*, p. 157; 1913, p. 104), does not belong here to judge by Hansgirg's original type (1885, p. 230). This possesses a gullet, as do other forms described by Zimmermann (1924, p. 8) and by Skuja (1939, pl. V, fig. 7) under the same name (cf. p. 147).

The state of affairs is similar in *Rhodomonas*. *R. baltica* was first described by Karsten (1898) as the type of the genus. He mentions the gullet. It was observed also by Zimmermann (1924, p. 4) and by Kylin (1935, p. 2). Zimmermann is of the opinion that cryptomonads without a gullet are of very doubtful validity, and that some investigators may have overlooked it. He points to the fact that most of the forms which are stated to possess no gullet are small in size.

This is the case also in *Cryptochrysis*. Knowing the difficulty of observation in cryptomonads and the occasional absence of trichocysts which alone make the outline of the gullet visible, I incline to agree with Zimmermann in his scepticism concerning the existence of cryptomonads without a gullet, the more so as nobody has observed such forms during the 20 years since Zimmermann's paper.

The simplicity of morphological structure, attributed to some forms, is not always convincingly proved. *Hillea fusiformis* Schiller (1925–6, p. 87), 'an incompletely known form recorded from the Adriatic, possibly represents a relatively primitive type. It lacks the marked furrow of other forms, its place being taken by a shallow depression confined to one surface' (Fritsch, 1935, p. 655). It seems, however, that Schiller used preserved material (formalin or sublimate, 1925–6, p. 59), which in this delicate group by no means preserves the original shape.[2] Indeed, the figures rouse the suspicion that they may represent badly fixed material of a form similar to *Rhodomonas*. Compare those reproduced by Fritsch (1935) with those of *Rhodomonas lens* Pascher & Ruttner (Pascher, 1913, p. 103). I may be mistaken, but I would not venture to establish a new genus on such uncertain evidence.

[1] *Rhodomonas rubra* Geitler (1922).

[2] Schiller's figures and descriptions are rather poor, so that scarcely any of the many new species could ever be recognized. His lack of accuracy is borne out also by his first taking *Hillea* for a *Chlamydomonas* (1913, p. 625).

A third group of Cryptophyceae, apart from Cryptomonadineae and Phaeocapsineae, are the Nephroselmidaceae of Pascher. Though it is difficult to judge without personal knowledge of the forms concerned, I append some critical remarks which may help others to obtain further information. The Nephroselmidaceae are characterized by a bean-shaped body with two laterally attached flagella. There are only three monotypic genera,[1] *Nephroselmis* with *N. olivacea* Stein, *Protochrysis* with *P. phaeophycearum* Pascher and *Sennia* with *S. commutata* (Senn) Pascher. *Nephroselmis* has, since its first discovery, been found by Scherffel (1912, p. 114), Pascher (1913, p. 111) and Prescott (1930, p. 32). Scherffel believes it to be a member of Volvocales, and in spite of the authority of Pascher, who does not mention Scherffel's paper, the reference of *Nephroselmis* to the Cryptophyceae does not seem to be definitely established. Prescott does not give any new information.

*Protochrysis* has been found only once (Pascher, 1911*a*, p. 192; 1912*a*, p. 182). In its organization it is as unlike other Cryptophyceae as *Nephroselmis*. Thus only these two have two alternately contracting vacuoles. *Sennia*, the third genus of Nephroselmidaceae (Pascher, 1912*a*, pp. 180, 182) is so small that its structure can hardly be recognized.

Pascher's conclusion that these three genera should be grouped as a family of Cryptophyceae may be correct, but it should be confirmed by a more detailed investigation.

### (3) *Colour*

One striking attribute of Cryptophyceae is the diversity in colour which surpasses that seen in Cyanophyceae and Dinophyceae, while Chrysophyceae and Bacillariales show it to a much slighter degree. In all these classes a single hue predominates. In Cyanophyceae it is bluish green, in Dinophyceae, Chrysophyceae and Bacillariales golden brown, while in Cryptophyceae the majority seems to be brown, but many have a colour which is not readily defined, having something of green, brown and grey in it. The best description is perhaps olive green. Cryptophyceae, however, include also blue green, greenish blue, yellow, reddish grey, red and colourless species.

The taxonomic bearing of this variability in colour is not clear as yet. Pascher (1912*b*, p. 189) says, that Cryptophyceae, like Chrysophyceae, are generally brown, but more green in waters rich in humic acid or other organic substances, and that individual reddish and blue-green variants are always found in a large material. There are, however, according to him, also forms which have fixed their blue-green or red hue. Repeating the same suggestion he adds later (1923, pp. 313–14) that certain cryptomonads (*Cryptomonas*, *Cryptochrysis*) are liable to great variability in hue. What does that mean? Is Pascher of the opinion that some of these forms are modified by external influences in the same direction as others are transformed by mutation? If so, we should try to differentiate both phenomena, in order to find a better basis for the use of pigmentation in taxonomy. So far, however, I have not found anything pointing in this direction. Apart from the changes in tint due to nutritional exhaustion, colours of species seem to be constant and populations uniform, if taxonomically homogeneous.

In fact the colour may change in the same species or strain and even in the same cell. This is most evident in blue-green forms which tend to fade into fallow brown or olive green in old cultures. The change might easily escape notice in nature when the

[1] *Nephroselmis marina* Schiller (1925–6, pp. 87, 91) was so incompletely described, that its significance cannot be judged.

original colour would be unknown.[1] Probably every species is liable to undergo such alterations of colour. Reddish brown forms for example become yellow. These colour changes are probably due to the same causes as in Cyanophyceae (Magnus & Schindler, 1912; Pringsheim, 1914, p. 73) and are altogether comparable with them. The faded cells of old cultures are by no means dead, but may remain motile and can be restored to their previous colour by adding a suitable nutritive solution.

Changes in colour are likely to be due also to other causes, especially the red shades found in Cryptophyceae inhabiting the deeper regions of lakes (Geitler, 1922; Pascher, 1923), but at present nothing definite is known about them.

Differences in colour have been used to characterize not only species but even genera, although such a practice has not been employed in any other group of organisms. Certain genera of Cryptochrysideae are distinguished by Pascher (1913, p. 101) merely on the basis of colour, *Cryptochrysis* being brownish, greenish or reddish, *Rhodomonas* red and *Chroomonas* blue-green. As seen from this, the differences are not even clean cut.

Hansgirg (1885, p. 230) was the first to create confusion by the use of colour as a generic character in Cryptophyceae. It was he who established the genus *Chroomonas* for Cryptomonads with blue-green chromatophores. His type species, *C. Nordstedtii*, is insufficiently described to enable one to judge whether forms referred to this species by more recent investigators are really identical with it. A species described and figured by Skuja (1939, p. 91, pl. V, fig. 7), who does not refer to Pascher's definition of the genus *Chroomonas* as lacking a gullet, resembles Hansgirg's form in possessing it. Skuja's form would, according to Pascher, be a *Cryptomonas*. The same remarks apply to two other species of '*Chroomonas*' described by Lackey (1936, p. 494), Carter (1937, p. 56) and Skuja (1939, p. 91). These authors, like Hansgirg, thus rely solely on the blue-green colour as a means of generic distinction. This custom should be abandoned. Geitler upholds Pascher's diagnosis and only refers species, in which he could not observe a gullet, to *Chroomonas* (*C. caudata*, 1924), while he includes a species with blue-green chromatophores but possessed of a gullet in *Cryptomonas* (*C. coerulea*, 1922, p. 689). Pascher (1913) does not describe any blue-green species of *Cryptomonas*.

### (4) *Apochlorosis*

A few forms are altogether colourless owing to the absence of chromatophores and their pigments. Since they have presumably originated from chlorophyll-containing species, they are designated apochlorotic. Their taxonomic position is not yet clear.

Pascher (1913, p. 107) separates the colourless Cryptophyceae from the others and groups them in the suborder Chilomonadeae. The three genera (*Chilomonas*, *Cyathomonas*, *Phyllomitus*) represent widely differing types within this comparatively uniform class. *Chilomonas* is an apochlorotic parallel to *Cryptomonas* and is dependent on dissolved organic substances (Loefer, 1934; Pringsheim, 1921, 1935), which are utilized also by certain of its coloured relatives. *Cyathomonas*[2] and *Phyllomitus* are highly

[1] Cf. however, Geitler's (1924*a*, p. 360) description of the change in colour in *Rhodomonas*.

[2] Skuja (1939, p. 95) failed to find food particles inside the cell and therefore believed his form to be saprophytic. Yet two apparently identical strains could not be grown without bacteria and showed plenty of food vacuoles in every cell of the thriving cultures. The observation, repeatedly stressed by Skuja in this, otherwise excellent, paper that forms hitherto considered to be holozoic were found without food particles, should not be used to draw conclusions concerning the manner of nutrition. Food vacuoles may last only for a short time, their contents being thrown out under unfavourable conditions.

specialized holozoic forms. They differ markedly from *Chilomonas* as well as from one another. *Cyathomonas* has two almost equal flagella and ingests bacteria, while *Phyllomitus* has one swimming and one trailing flagellum and ingests starch grains and other vegetable residues. *Phyllomitus* is rightly placed in the new family Kathablepharidaceae, established by Skuja (1939, p. 96), who retains *Cyathomonas* among the Cryptomonadaceae. It would, however, be advisable to found for this genus a special family, Cyathomonadaceae, embracing a few closely related forms, although *Cyathomonas* is the only representative so far described.

### (5) *Number of chromatophores*

Apart from those Cryptophyceae which lack chromatophores most species are stated to possess one, two or several chromatophores, two being the commonest number. When there are one or two, they appear as thin sheets or lamellae, often lobed or fringed, and usually covering the greater part of the inner surface of the periplast. It is not always easy to decide whether there is only a single deeply incised chromatophore or whether two are present. When this is so, one is larger and seems sometimes to consist of two parts, which may be completely separated from one another.

A larger number of discoid chromatophores has been recorded in two instances, viz. *Cryptoglena americana* Davis (1894), renamed *Cyanomonas americana* by Oltmanns (1904, p. 30) and *Cryptochrysis polychrysis* Pascher (1913, p. 102) (cf. p. 146). Forms resembling the former were recorded by Pascher (1913, p. 104), although he could not furnish diagnoses owing to insufficiency of material.

Without knowlege of the circumstances under which these forms were observed and investigated, no criticism can be conclusive, but there are two sources of error. Large chromatophores are sometimes lobed to such a degree that fragments become detached, especially under harmful influences. The other, more serious, source of error is an optical illusion by which I myself was twice misled (cf. also Carter, 1937, p. 53). When the chromatophores are only faintly coloured and light is concentrated by the lens-like action of the starch grains, these alone appear coloured, giving the impression of small round chromatophores. Only the best optical equipment and illumination, which may not always have been available in the field, can dispel this optical illusion and elucidate the actual nature of the internal structures. Species stated to have discoid chromatophores therefore require careful re-examination and more exact description. Among scores of strains of Cryptophyceae I have not found one with discoid chromatophores.

My suspicion appears to be justified by the text of Davis's publication (1894, pp. 97, 98). He writes: 'Inside the cell are from six to ten disk-shaped bodies, arranged around the periphery of the cell. These bodies are not pyrenoids, nor are they amorphous albuminous matter, for they are readily destroyed by a dilute potassic hydrate solution after long treatment in mercuric chloride in absolute alcohol. For this reason it seems as though these bodies are true chromatophores, although the blue-green colour seems to fill almost the entire cell, only the end which bears the cilia being hyaline.'

From this it is clear that Davis mistook starch grains—which he does not mention—for chromatophores. The shape, position and chemical behaviour of his 'chromatophores' are exactly those to be expected of starch grains. Davis's remark, that the morphology of the cell is simpler than in *Cryptomonas*, is likewise not conclusive.

Copious starch content must have interfered with observation of the finer structure of the cell. Nothing definite can therefore be said about this form.

The other species which has been described as being possessed of more than two chromatophores is *Cryptochrysis polychrysis* Pascher (1912*b*, p. 157; 1913, pp. 101–2). Nobody has observed it since its first discovery. It is rather small. Pascher's figure does not disclose anything pointing to a confusion with starch grains.

It seems that Pascher does not attribute much importance to the possession of two or several chromatophores in Cryptophyceae. Otherwise he would not refer *Cryptochrysis commutata* Pascher with two elongate chromatophores to the same genus as *C. polychrysis* with several discoid ones, while separating *Chroomonas* and *Cyanomonas* because of the same difference.

A source of error concerning species of Cryptophyceae with more than two chromatophores may also originate from the existence of flagellates which contain blue-green symbionts in their otherwise colourless cells. Pascher (1929, p. 409) admits that such a form, named by him *Chroomonas gemma* (Playfair) Pascher, may really be a symbiontic consortium. But he does not refer to his comment (1913, p. 104) on two species of '*Cyanomonas*' which, according to him, require accurate investigation.

I personally do not think that endosymbiontic Cyanophyceae could be confused with chromatophores by an experienced observer. On the whole, however, the existence of Cryptophyceae with several chromatophores needs further investigation.

The possession of one or two chromatophores respectively has not been used to distinguish genera. According to Pascher *Cryptomonas* has one or two chromatophores. A single chromatophore cannot be used as a taxonomic feature, because intermediate forms with more or less lobed chromatophores can be found. Fritsch (1914, pp. 347–9) did not even establish a new genus for his *Cryptomonas anomala*, although its chromatophores have a disposition the reverse from that of the normal, which may be regarded as a more essential difference than an imperfect incision during cell division.

## III. Conclusions

In view of the facts mentioned above and the defects displayed by the existing scheme I am of the opinion that the classification of Cryptophyceae should be adjusted to meet the needs, both of a theoretical taxonomy and of practical identification. The difficulty, arising from the fact that some of the features for a natural grouping are hard to observe, must be overcome. It is impossible to employ one system of classification for one purpose and a different one for another. For the time being a satisfactory grouping of Cryptophyceae can scarcely be achieved for lack of essential knowledge. There is reason to hope, however, that this will, in course of time, become possible, as a result of extensive examination of numerous strains, which alone can lead to further progress.

In the meantime I suggest a subdivision confined to the following five families:

(1) Cryptomonadaceae, in the sense of Pascher and Fritsch.

(2) Cryptochrysidaceae Pascher. Gullet lacking, trichocysts situated in a shallow furrow; chromatophores, contractile vacuole and flagella as in Cryptomonadaceae.

(3) Cyathomonadaceae, nov.fam. Chromatophores absent, holozoic. Body flattened, front end transversally truncate, with a single ring of trichocysts in a shallow excavation. Two flagella of nearly equal length and contractile vacuole as in the two preceding families.

(4) Kathablepharidaceae Skuja. Chromatophores absent, holozoic; characterized by the ventral furrow, sometimes running spirally round the body, and by two diverging flagella, the one directed forwards, the other trailing behind; contractile vacuoles at or beneath the middle of the cell; individuals exhibiting jerky backward movements like those seen in the first two families.

(5) Nephroselmidaceae Pascher. Body bean-shaped; flagella laterally inserted.

If the absence of a gullet must be discarded as a means of taxonomic differentiation, and if we abandon the use of colour as a generic feature, we would be obliged to refer all pigmented forms, with the exception of Nephroselmidaceae, to *Cryptomonas*. We should then attempt to find new features admitting of generic differentiation, if there are really as many independent species as a survey of the literature and observation in nature seem to indicate.

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# SOME EXAMPLES OF CORRELATION BETWEEN STIPULES AND LATERAL LEAF TRACES

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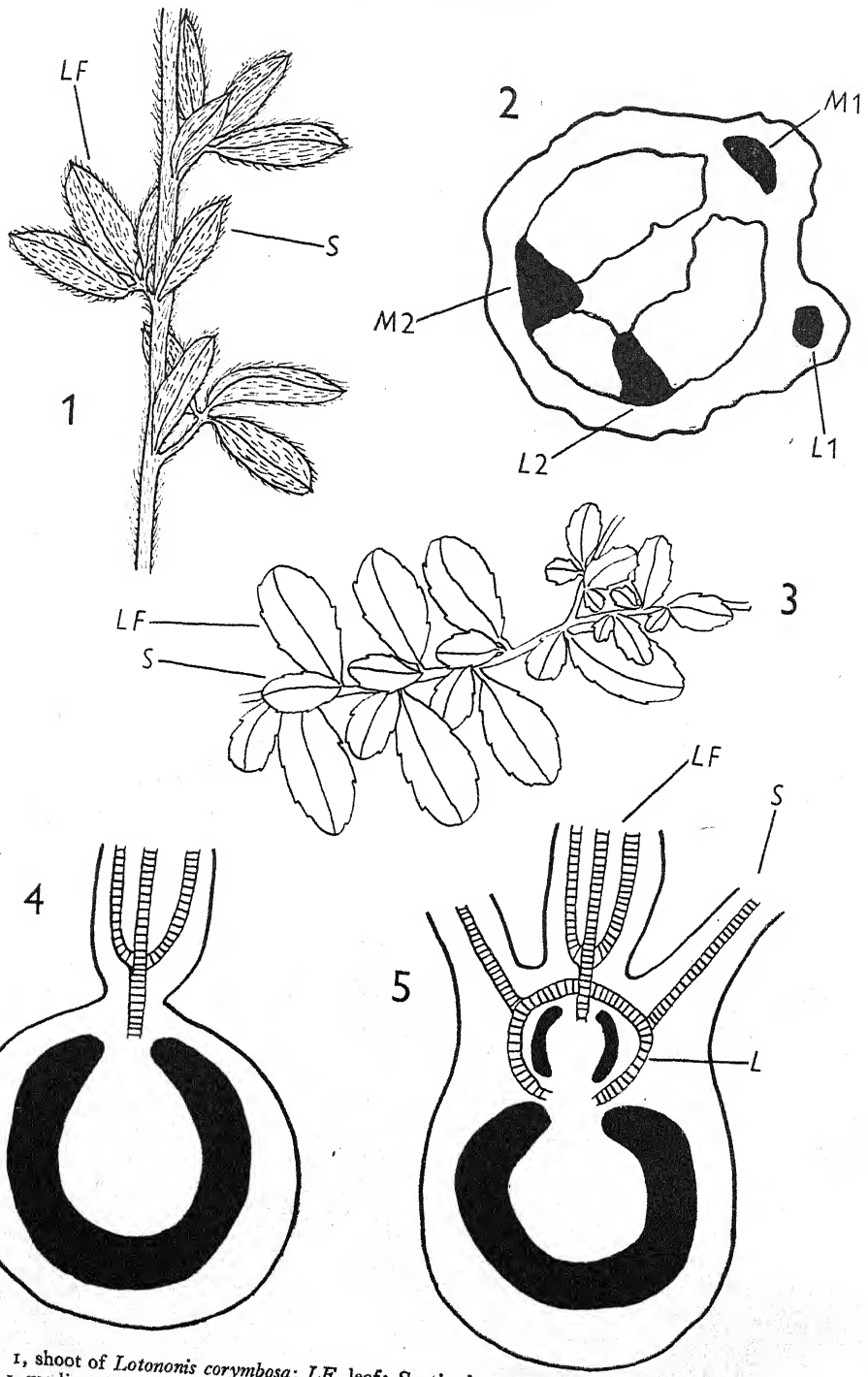
(With 5 figures in the text)

Colomb appears to have been the first to state the important rule that the vascular supply to a stipule is always a branch from that of the associated leaf (Colomb, 1887). The definition (Sinnott, 1914) of three types of nodal structure and the demonstration of their systematic importance opened the way to a further generalization. It was shown in the same year (Bailey & Sinnott, 1914) that unilacunar forms are mostly exstipulate, trilacunar ones mostly stipulate, while most multilacunar forms have sheathing leaf bases. Some particularly elegant examples of this kind of correlation have recently come to light.

*Lotononis corymbosa* Benth., a shoot of which is shown in Fig. 1, has trifoliolate petiolate leaves arranged distichously. Each leaf has a single large stipule (*S*, Fig. 1), resembling a leaflet in form and texture. All the stipules lie on the same side of the stem, so that the shoot has definite stipulate and exstipulate faces. Transverse sections such as that shown in Fig. 2 reveal that the two ranks of leaves are not diametrically opposite, the angular divergence being greater on the stipulate side than on the exstipulate. Each leaf receives two traces, one of which (*M* 1, Fig. 2) clearly corresponds to the median trace of a trilacunar node, since the traces to the axillary shoot arise from the margins of its gap. The other foliar trace (*L* 1, Fig. 2) is to be regarded as lateral, and gives off a branch to the stipule before entering the leaf. The Leguminosae, to which *Lotononis* belongs, are prevailingly trilacunar and symmetrically stipulate, so that there is no reason to doubt that the peculiar bilacunar condition is the result of suppression of one stipule and of the corresponding lateral trace. The genus offers considerable scope for further investigation, as there are both exstipulate species and species with two equal stipules. There appears to be a definite correlation between stipular development and phyllotaxy, the species with one stipule being distichous, those with two spiral. No exstipulate form has been examined.

A close parallel to the condition just described is to be found in *Azara microphylla* Hook., one of the Flacourtiaceae, a shoot of which is illustrated in Fig. 3. Except that the leaves in this case are simple and sessile, the structure does not differ from what has been described for *Lotononis corymbosa*. The plant is an evergreen shrub, and the twigs are placed with their stipulate faces upwards, the large stipules (*S*, Fig. 3) playing a conspicuous part in the formation of the leaf mosaic.

*Acacia verticillata* Willd. has attracted a good deal of attention on account of the specialization of its shoots. The twigs bear acicular phyllodes arranged in whorls; the verticillate phyllotaxy is inconstant, a single phyllode or a small group often appearing between two whorls. Often there is a curious tendency to the production of half-whorls. Only a limited number of phyllodes have axillary shoots, never more than one in a




Figs. 1–5. 1, shoot of *Lotononis corymbosa*: *LF*, leaf; *S*, stipule. 2, transverse section just below a node: *M*1 and *L*1, median and lateral traces; *M*2 and *L*2, those of the next leaf above. 3, shoot of *Azara microphylla*, seen from above. 4, diagram of vascular supply to a sterile phyllode of *Acacia verticillata*, xylem cut transversely black, that cut longitudinally cross-shaded. 5, vascular supply to a fertile phyllode: *L*, lateral foliar trace.

whorl, and not by any means one in every whorl. This condition gave rise to the supposition that only the phyllodes with axillary shoots, the so-called *fertile* phyllodes, were genuinely foliar, the others being of the nature of stipules. This hypothesis was put forward by Hofmeister (1868), who drew a comparison with the well-known composite whorls of Rubiaceae-Stellatae. According to Reinke, a similar notion appears in a dissertation by Kaufholz in 1888. Both Reinke and Goebel rejected Hofmeister's interpretation (Reinke, 1897; Goebel, 1905), but their arguments are based purely on external morphology, and the internal structures seem never to have been investigated.

The fertile phyllodes are provided with paired stipules which, though small, hardly merit the description of 'rudimentary' applied to them by Goebel. Considering that the phyllodes to which they belong are not more than about 15 mm. long, they are reasonably well developed, and each receives a distinct vascular supply. The *sterile* phyllodes, on the other hand, are normally devoid of stipules.

Transverse sections show that every phyllode receives its own independent vascular supply from the stele, so that the hypothesis of false whorls comparable with those of *Galium* is obviously ruled out. Each sterile phyllode is served by a single trace, which divides into three bundles on entering the base of the organ, as shown in Fig. 4. Each fertile phyllode has, in addition, two lateral traces (*L*, Fig. 5), each of which gives off a branch to the stipule before fusing with the median and running into the phyllode. The lateral traces are separated from the median, as shown in Fig. 5, only by the traces to the axillary bud. As the sterile phyllodes show not the slightest vestige of bud traces, it seems not impossible that the reduction to a single foliar trace may be a direct mechanical consequence of the omission of the bud supply.

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# THE GENUS *CHYTRIDIOCHLORIS*

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(With 54 figures in the text)

For some time after its discovery the genus *Harpochytrium* was considered to have chytridiaceous affinities: in 1899 Gobi expressed doubts as to this opinion, while Wille (1903) thought that the plants were colourless saprophytic algae. Scherffel (1926) described several species of *Harpochytrium* in some detail, including *H. viride*, a plant of somewhat similar form to *Harpochytrium* but possessing a chromatophore; thus the algal affinities of the genus appeared to be established. In his treatment of *Harpochytrium* Pascher (1938) divides the genus into two subgenera, *Chytridiochloris* for the reception of the green species, *Harpochytrium viride* and *H. Scherffelii*, and *Harpochytrium* for the non-pigmented species. The present author (Jane, 1942) described plants of *Harpochytrium* which closely resembled *H. tenuissimum* Korsch: after carrying out numerous tests with a view to determining the affinities of the plant, he was unable to decide upon its systematic position, and suggested the desirability of raising Pascher's two subgenera to generic rank, *Chytridiochloris* for the reception of the pigmented species, which are, without question, xanthophycean algae, and *Harpochytrium* for the colourless species, the systematic position of which he regarded as uncertain and whose affinities with the pigmented species he considered non-proven, a conclusion justified by a subsequent study of these two genera. As a consequence, the so-called green species of *Harpochytrium* are removed to the genus *Chytridiochloris*, and the results of the detailed examination of *C. viridis* may now be given.

*C. viridis* is probably not uncommon in mountain pools in Snowdonia, where the present author has collected it from four localities, the richest one being a new peat cutting at Nant y Benglog, Capel Curig. One or two plants were also found at Criccieth. Apart from these Caernarvonshire localities, a few plants were collected from a temporary pool on Llandegfan Common, Anglesey, in which, in spite of the low altitude, the algal flora is essentially montaine.

## Morphology

(*a*) *Size and shape. C. viridis* is somewhat variable in shape, and considerably so in size; there is also much variation in its position in relation to its host, by which general term nothing more than the plant on which *Chytridiochloris* is lodged is meant, with no implication of parasitism.

Mature cells are always curved, although the curvature may vary considerably (Figs. 8, 11): thus at times the distal part of the cell lies more or less at right angles to the proximal part (Figs. 13, 22), in others the curvature may be even greater (Figs. 16, 32). The cells usually become curved early in life (Fig. 50).

Apart from the two extremities, the cell is usually of even diameter throughout (Figs. 10, 15), but sometimes broader toward the apical end (Figs. 27, 29). Plants such as those

shown in Figs. 18 and 19, in which the cell is broadest at or near its centre, may be regarded as exceptional. At the base the cell is nearly always narrower than at the apex; as a rule the basal taper is fairly sharp (Figs. 4, 14), but it may be more gradual (Figs. 10, 35). At most, the base is bluntly pointed (Figs. 4, 26), but often it is rounded (Figs. 13, 32) and at times is almost without taper in this region (Fig. 16). The apex is always broadly rounded. There is no basal differentiation into a stalk and foot region, but the rounded base fits into a truncated cone, which, owing to its transparency, may easily escape detection. This cone (Figs. 10, 21) is presumably mucilage, but its precise nature could not be determined; it is faintly coloured violet by ruthenium red, it is indifferent to methylene blue and but slightly differentiated by trypan blue.

The range of dimensions of the plants is wide. Thus the specimen drawn in Fig. 24 with a length of 41$\mu$ and a diameter of 4·5$\mu$ is shown on the same scale as that in Fig. 25, which measures only 16$\mu$ and has a diameter of 2·5$\mu$: since both cells have dehisced sporangia, it may be assumed that both have reached maturity. Cells have been noted as long as 50$\mu$ (Fig. 31), although plants with a length exceeding 40$\mu$ are rare. The diameter varies from a little under 2·5$\mu$ to nearly 6$\mu$, but plants with a diameter in excess of 4·5$\mu$ are uncommon. Scherffel (1926) met with broader cells; he gives the dimensions as up to 30$\mu$ long and up to 8$\mu$ thick.

*C. viridis* may coil around the host cell (Figs. 29, 32), to which it is not usually closely adpressed; it does not invariably curl round the cell transversely; often it grows away from the host (Figs. 22, 24) so as to be almost erect (Figs. 12, 23).

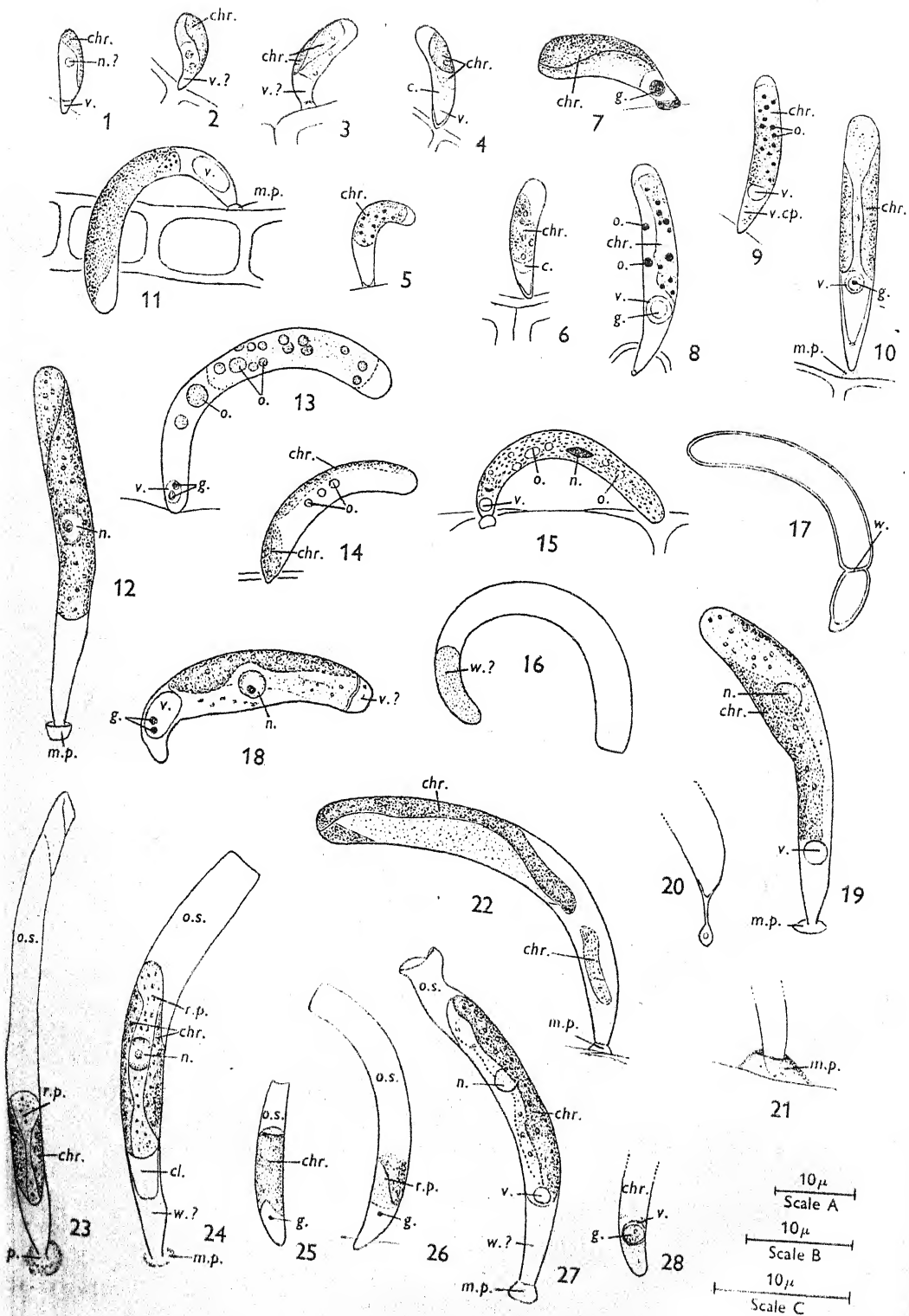
The size of *C. Scherffelii* Pascher differs little from that of *C. viridis*. To judge from the three plants which Pascher figures (Figs. 52–54), the cells have much the same shape as the more strongly curved ones of *C. viridis*; the examination of a larger number may reveal as much variation as obtains in *C. viridis*. The apex of *C. Scherffelii* is rounded and the base shortly tapered: apart from this, the cells are cylindrical; the base may end in a minute stalk and foot, but on this point Pascher (1938) is not clear. The plants appear to be in close contact with the host filament, around which they lie more or less transversely.

(*b*) *Cell wall.* In *C. viridis* reactions of the wall to cellulose tests are negative, and treatment with ruthenium red or methylene blue gives no indication of the presence of pectic substances. The wall is not appreciably stained by picro-nigrosin, a possible indication that it does not contain chitin; more elaborate tests failed to give a certain positive reaction for this substance.

An aqueous solution of congo red stains the walls a faint pink, which is somewhat intensified by previous treatment with hot 20 % potash. The hot potash treatment produced a slight swelling of the wall, but yielded no evidence that the wall was laminated nor that it was composed of two overlapping pieces: however, in so thin a wall, the laminae might not be resolved with the highest magnifications, even when swollen.

The cell wall is remarkably resistant to cold concentrated sulphuric acid—much more so than that of its host—and to a 40 % aqueous solution of chromic acid. The sole visible effect of chromic acid is the detachment of the plant from its host.

While the cell wall is very thin and delicate, it often shows some thickening at the base of the cell (Figs. 4, 10, 17). At times the base of the cell appears to be remarkably thickened (Figs. 16, 24, 27), a feature noted by Scherffel (1926); whether this is wall thickening is not certain; the basal region is often filled with a clear, greyish homogeneous material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(cf. Figs. 24, 27), which appears to be continuous with the wall, and which might be taken for an extension of the wall were it not for the fact that it seems but little affected by stains which colour the cell wall. It is difficult, however, to regard this material as anything other than the greatly thickened wall, a view which is strengthened by the plant shown in Fig. 16; this plant was dead and devoid of contents, and the basal region seemed clearly to be part of the cell wall.

(*c*) *Abnormalities.* Few abnormalities were encountered in plants of *C. viridis*. One plant only was observed in which the base tapered into a rather long, thin stalk, ending in a knob-like foot, which was, possibly, hollow (Fig. 20). Likewise the plant shown in Fig. 17, with a septum toward the proximal end of the cell, is unique in the author's experience, although two reproducing plants were observed which probably possessed septa (Figs. 30, 35).

Apparent anomalies of chromatophore structure are dealt with later.

(*d*) *Protoplast.* The cells of *C. viridis* possess a single large green chromatophore, which commonly occupies about the distal two-thirds of the cell (Figs. 10, 24); it may reach to the cell apex (Fig. 22), but this is not usual. The chromatophore is shaped like a very tall collar (Figs. 10, 24) or sometimes like an elongated saddle (Figs. 18, 27). Apart from its length, it often bears a striking resemblance to the chloroplast of *Ulothrix* (Figs. 10, 23), but sometimes it covers only half, or even less, of the circumference of the cell. The back of the chromatophore almost invariably occupies the convex side of the cell.

In only one plant was the chromatophore found to extend to the base of the cell (Fig. 14), and in this it was deeply constricted about one-third of its distance from the proximal end, or possibly divided into two at this point. In another plant (Fig. 22) two chromatophores were certainly present, a large distal one and a smaller proximal one.

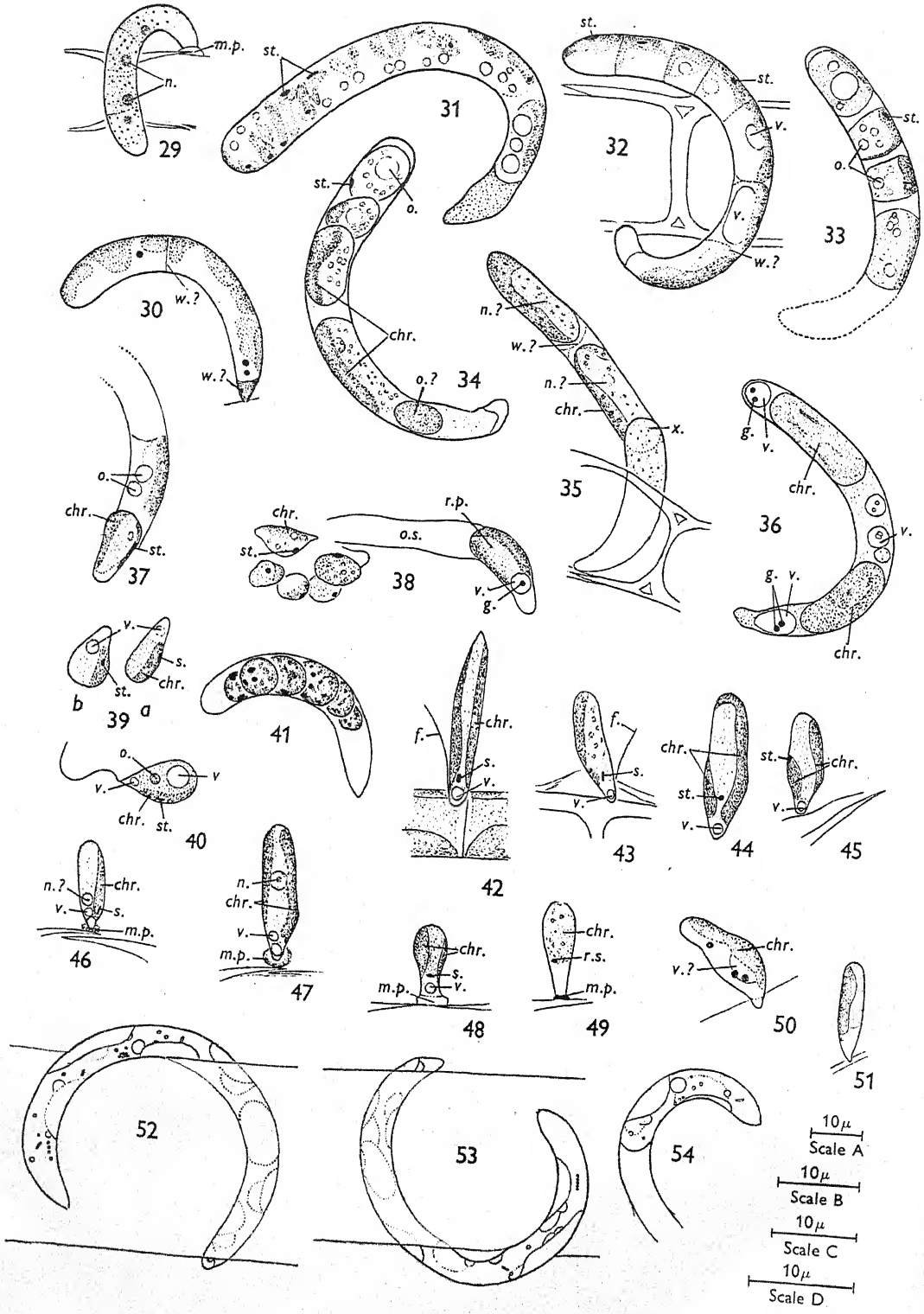
*C. Scherffelii* has several relatively small chromatophores, which are plate-like or trough-like, and distributed more or less regularly through the cell except at its proximal end. Its chromatophores are not orientated in any special manner.

Occasional plants of *C. viridis* may be found with several chromatophores (Figs. 31, 32). In *C. viridis*, however, these chromatophores are linearly arranged with their backs directed to the convex side of the cell; their increased number is due to the division of the cell contents before spore formation. In such plants a thin, elongate, reddish brown stigma may be associated with each chromatophore (Fig. 32): stigmas are not present in the vegetative cells and are associated with zoospore formation.

As is not uncommon in the Xanthophyceae, the limits of the chromatophore of *C. viri-*

**Legends to Figs. 1–28**

Figs. 1–28. *Chytridiochloris viridis*. Figs. 1–7. Sporelings and young plants; scale B, except Fig. 7, which is scale C. Figs. 8–15. Older plants, that shown in Fig. 13 has been stained to show the oil; that shown in Fig. 15 was fixed with Carnoy's fluid and stained with Heidenhain's haematoxylin; all scale B, except Fig. 11, which is scale A. Fig. 16. Empty cell in which, probably, all the contents were used in spore formation; scale B. Fig. 17. Cell after potash treatment; this cell is unique in showing a transverse wall; scale B. Figs. 18, 19. Mature plants, both broad in relation to their length; scale B. Fig. 20. Base of plant with a unique stalk and foot; scale C. Fig. 21. Typical base of plant; scale C. Fig. 22. Plant with two chromatophores; scale B; cf. with Fig. 14. Figs. 23–27. Plants with empty sporangia; in Figs. 23 and 26 the zoospores have been recently shed, in Fig. 27 the residual protoplasm has started to grow again; both in Figs. 23 and 27 the old sporangia show signs of collapse; all scale B. Fig. 28. Base of plant showing vacuole with large granule; freehand. *c.* finely granular cytoplasm; *chr.* chromatophore; *cl.* clear area; *g.* granule; *m.p.* mucilage pad; *n.* nucleus; *o.* oil; *o.s.* old sporangium; *r.p.* residual protoplasm; *v.* vacuole; *v.cp.* vacuolate cytoplasm; *w.* wall.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s.
m.p.
46
n.
chr.
v.
m.p.
47
chr.
s.
v.
m.p.
48
chr.
r.s.
m.p.
49
chr.
v.?
50
51
52
53
54
10μ
Scale A
10μ
Scale B
10μ
Scale C
10μ
Scale D

*dis*, and especially the lateral limits, are not always easy to determine by reason of its pale colour. The colour itself is not of the yellow-green which might suggest xanthophycean affinities.

Chromatophore apart, the most prominent feature in many, but not all cells of *C. viridis*, is a large spherical (Fig. 19) or rarely ellipsoidal (Fig. 11) vacuole, which lies in the cytoplasm below the chromatophore. This vacuole sometimes contains a single granule of varying size (Figs. 8, 10, 28), or several small granules (Figs. 18, 36) which may exhibit brownian movement. Sometimes there are no granules in the vacuole (Fig. 19) and in those few plants in which the vacuole is not discernible (Figs. 12, 22), a granule may be present (Fig. 25) in its position. Normally the cytoplasm surrounding the vacuole is clear and transparent, but occasionally it may be vacuolated (Fig. 9). Pascher (1938) refers to a ball of colourless substance, perhaps leucosin, which occurs in this position in the cell of *C. viridis*: possibly the body to which he refers (cf. Scherffel, 1926, fig. 20*b*) is the vacuole, for if the vacuole were free from granules an error in interpretation might easily be made. Nevertheless, it is possible that in the figure referred to above the spherical, vacuole-like body is a solid granule, comparable with that shown in my figures.

Conspicuous vacuoles are rarely found in other parts of the cell, although they have been noted at the apex and also in the centre of the cell (Fig. 36). Very occasionally the segments of the cell which are to become zoospores (Fig. 32) possess large vacuoles.

Apart from the chromatophore and vacuole, the cell contents are rarely conspicuous, being usually masked by the chromatophore. In the chromatophore region there are numerous small refringent granules, of which it has not been possible to determine the nature: some of them are darkened when the plants are boiled for 5 min. in 10 % silver nitrate, so that they evidently have reducing properties. Further it was noted that some of the granules assume a faint red colour when cells are placed in a 1:2500 aqueous solution of ruthenium red. This might indicate the presence of pectic substances, but since methylene blue gave no positive reaction for pectin the evidence is weak. Some of the granules are certainly fat, and often rather large oil globules are present (Figs. 13, 15), but not readily seen until stained with an appropriate fat reagent.

In *C. Scherffelii* Pascher states that oil drops and solitary protein crystals occur. In the plants which he figures there is no trace of a proximal vacuole similar to that which has just been described in *C. viridis*.

**Legends to Figs. 29–54**

Figs. 29–51. *Chytridiochloris viridis*. Fig. 29. Plant showing segmentation in preparation for spore formation; stained Feulgen method after Carnoy fixation; scale B. Fig. 30. Segmenting plant with wall or membrane across middle of cell; scale D. Fig. 31. Plant with numerous small segments in sporangium; scale D. Fig. 32. Plant with segmenting sporangium; this is a more usual type than that shown in Fig. 31; scale D. Figs. 33, 34. Plants with three spores each; scale D. Fig. 35. Plant with two large spores, probably aplanospores, separated by wall or membrane; scale B. Fig. 36. Plant with two large spores; the middle part of this plant was somewhat obscured by an overlying alga; note apical and central, as well as basal vacuoles; scale D. Fig. 37. Apical part of plant with zoospore at mouth of sporangium; scale D. Fig. 38. Plant which has just shed its zoospores; scale B. Fig. 39 *a*, *b*. A zoospore from the group shown in Fig. 38; drawing 39*b* was made 2 min. after 39*a*; scale B. Fig. 40. Zoospore with a large posterior vacuole, an unusual feature; scale B. Fig. 41. Plant with six aplanospores; scale D. Figs. 42, 43. Zoospores which are just attaching themselves to host plants; scale D. Fig. 44. Unattached zoospore; flagellum was not observed; scale D. Fig. 45. Attached zoospore; scale D. Figs. 46–51. Very young sporelings. Fig. 46, scale C; Figs. 47, 49–51, scale D. *chr.* chromatophore; *f.* flagellum; *g.* granule; *m.p.* mucilage pad; *n.* nucleus; *o.* oil; *o.s.* old sporangium; *r.p.* residual protoplasm; *r.s.* remains of stigma; *st.* stigma; *v.* vacuole; *w.* wall; *x.* plant bent over in this region.

Figs. 52–54. *Chytridiochloris Scherffelii* (after Pascher). Approximately scale A.

The cells of *C. viridis* contain a single nucleus which occupies a more or less central position, although it often lies toward the concave side of the cell. The nucleus is rarely seen in the living cell, nor are temporary fixatives like iodine in potassium iodide likely to reveal it. Permanent fixation and staining show it as a body, lenticular (Fig. 15), ellipsoidal (Fig. 12) or more or less spherical (Figs. 19, 24) in shape, and containing a single nucleolus. There is no information regarding the nucleus of *C. Scherffelii*.

## REPRODUCTION

The method of reproduction in *C. Scherffelii* is unknown. In *C. viridis* it is by zoospores and, apparently rarely, by aplanospores.

In the production of zoospores the nucleus divides, and the distal end of the cell, which now forms the sporangium, becomes segmented into a linear row of uninucleate blocks, which, by rounding off, produce the spores (Figs. 32, 33). Normally these segments do not exceed some half-dozen, although at times they number about fifteen and are then smaller (Fig. 31). Each segment contains a single, pale green, parietal chromatophore, which is saddle-shaped, with its back to the convex side of the cell (Figs. 32, 33); on this side also is a small, elongate, reddish brown stigma. Sometimes each block contains a number of fat globules (Figs. 33, 34), but often the cytoplasm contains a number of small granules and there are no obvious storage products (Fig. 32). Each block may contain a small or a large vacuole (Fig. 32).

In one plant, which was observed alive, seven zoospores had just been shed (Fig. 38); at first they were non-motile, but they soon showed a slight movement, and a little later a single flagellum was observed. Little could be made of the shape of the very pale chromatophore. The spores possessed a conspicuous lateral stigma and contained numerous small granules which were probably oil. At first the zoospores were very sluggish, moving slightly and then coming to rest; their shape did not remain constant, and one was observed to change to the fusiform shape characteristic of the old zoospore, and then rapidly to become pyriform. In this particular zoospore (Fig. 39 *a*, *b*), an anterior vacuole was visible, but the flagellum could not be seen; the changing shape was its most striking feature. One zoospore (Fig. 40) showed a large posterior vacuole in addition to the anterior one, but this is regarded as exceptional.

The older zoospores are fusiform (Figs. 42, 43) and more or less flattened along one side and convex on the other. They possess a collar-shaped chromatophore, which does not extend to the anterior end of the cell: anteriorly the protoplasm is clear and contains a prominent, non-contractile vacuole; this vacuole may lie in the protoplasm which is covered by the anterior end of the chromatophore. There is a fairly prominent, elongated stigma lying near the anterior end of the cell and usually along the convex margin. The zoospore has a rather coarse and fairly short flagellum. These older zoospores appear to have lost their power of changing their shape.

It is unlikely that the zoospore always assumes a fusiform shape at maturity, although the attached zoospore shown in Fig. 43 and the shape of the very young sporelings which still retain the zoospore characters (Figs. 44, 47) clearly indicate that this type may represent the ultimate shape of the spore. Nevertheless, the shape of such young sporelings as those shown in Figs. 45, 46 suggests that zoospores of the shape shown in Fig. 39 may also form new plants.

Attention has already been drawn to the variation in size of the zoospores. Consideration of plants which have a relatively large number of small segments (Fig. 31) is omitted since their further development was not followed. Of plants in which the segments of the sporangium are fewer, those shown in Figs. 32 and 33 show a clear difference in segment or zoospore size; thus between the uppermost spore in Fig. 33 and its two adjacent fellows the difference is sufficiently striking, as is the difference between the two lowest and the antepenultimate one in Fig. 32.

The marked variation in the size and shape of the zoospores of *C. viridis* is not its least curious feature. The phenomenon is paralleled in the allied genus *Chlororhabdion* (Jane, 1944). There is, however, no evidence that zoospores of two types are produced, for there are all gradations between large and small ones.

Normally at least two-thirds of the distal end of the plant goes to form the sporangium (Figs. 23, 26, 38), although a smaller part of the cell may be involved (Fig. 34). The old sporangium decays after the escape of the zoospores (Figs. 23, 27). Wall formation across the naked top of the residual protoplasm seems to occur late, but the wall is very delicate and its detection is difficult.

There is good reason to believe that on occasion the whole of the cell contents is used in the production of spores, for occasional cells are seen, entirely devoid of contents (Fig. 16): in most dead cells some trace of the contents remains, and there are some signs of decay at the distal end.

## Sporeling

The sporeling settles down on its anterior end (Figs. 42, 43). In its early growth it is erect (Figs. 44–49), of somewhat irregular contour, with a rounded apex and usually, a pointed base. Somewhat older sporelings tend to be wider toward the apex and to taper somewhat toward the base (Figs. 1, 2): a basal swelling (Fig. 50) is very uncommon. In the youngest sporelings the anterior vacuole of the zoospore is usually prominent (Figs. 44–48), and the stigma may still be visible (Figs. 46, 48). The secretion of the mucilage pad (Figs. 46–48) occurs early. The sporeling soon becomes curved; erect specimens (Figs. 44–49) are rare.

## Resting stages

Resting stages are unknown in *C. Scherffelii* and are rare in *C. viridis*. Two plants were seen which contained large spores (Figs. 35, 36), and another with much smaller spores (Fig. 41), all devoid of stigmas. There is little doubt that these bodies are aplanospores; there was, it is true, no trace of oil in the spores, but since oil is only revealed in *Chytridiochloris* by the application of tests, it does not follow that oil was absent.

It is, perhaps, remarkable, that so few aplanospores were found in the large amount of material examined, but it is to be remembered that when shed from the sporangium they would hardly be recognized as such. Unfortunately all attempts failed to induce aplanospore formation in culture.

In the plant shown in Fig. 35 there is a wall separating the two spores. This suggests that the whole of the contents of the sporangium may have been used in the formation of a single aplanospore, and that the two spores arose by a process analogous to the proliferation which normally occurs.

In connexion with what has been written above in regard to the possible conversion of the whole of the protoplasm of a plant into spores, this appears to have occurred in the plant shown in Fig. 41. The resemblance to a plant of *Characiopsis* with aplanospores is striking.

## HOST PLANTS

Pascher found *Chytridiochloris Scherffelii* on *Spirogyra*, while Scherffel's material of *Chytridiochloris viridis* was on *Microspora*. In the experience of the present author *Microspora* and *Geminella* are by far the commonest host plants for *Chytridiochloris viridis*, although it has been found rarely on other hosts. In cultures it is found not infrequently attached to glass.

## TAXONOMY

The present investigation has produced evidence to justify the removal of the so-called pigmented species of *Harpochytrium* from this genus, and confirms the author's tentative conclusions (Jane, 1942) that these plants are worthy of generic rank. They are therefore referred to the genus *Chytridiochloris*. *C. viridis* with its storage of fat, and its uniflagellate zoospores is, unquestionably, a xanthophycean alga, which Pascher has correctly referred to the Heterococcineae (family Characiopsidaceae). *C. Scherffelii*, superficially, at any rate, is so similar, that its generic association with *C. viridis* is justifiable. Chromatophore apart, the resemblances between *C. viridis* and *Harpochytrium* are superficial, viz. the tubular shape, the proliferating sporangia and the uniflagellate zoospore. It may well be that homoplasy affords a sufficient explanation of the similarity. There are two outstanding differences between the two genera:

(1) The uninucleate plants of *Chytridiochloris* as compared with the coenocytes of *Harpochytrium*.

(2) The attachment of the zoospore by its anterior end in *Chytridiochloris*, and by the tip of its flagellum in *Harpochytrium*.

There is, moreover, often a spherical basal vacuole in *Chytridiochloris viridis* which appears to have no counterpart in *Harpochytrium*, although at times plants of the latter genus may show a more irregularly shaped vacuole at the base of the cell.

In the possession of a proliferating sporangium *Chytridiochloris* is not unique in the Characiopsidaceae, for the allied *Dioxys* possesses a similar organ. The two genera, alone in the family, show sterilization of the lower part of the plant, producing as it were, a perennial habit in contast to the ephemeral plants of other members of the family, where the whole contents of the plant form zoospores. The newly described member of the Characiopsidaceae, *Chlororhabdion* (Jane, 1944), may be regarded, in this respect, as intermediate between these two methods, for while the whole plant forms zoospores, one, sometimes more, remains behind in the parent sporangium, to produce a new plant *in situ*.

## SUMMARY

1. The structure and reproduction of *Chytridiochloris viridis* (Scherffel) Jane = *Harpochytrium viride* Scherffel are described.

2. The plant is uninucleate and has a single, large, collar-shaped chromatophore; there is commonly a large vacuole in the proximal part of the cell; the storage product is fat.

3. Reproduction is by uniflagellate swarmers and by aplanospores. The plant has a proliferating sporangium.

4. *Chytridiochloris* is a member of the Xanthophyceae (Heterococcineae, Characiopsidaceae). There is no conclusive evidence that it is related to the non-pigmented species of *Harpochytrium*.

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# GROWTH AND HETEROCYST PRODUCTION IN *ANABAENA CYLINDRICA* LEMM.

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(With 2 figures in the text)

In the course of investigations on the nitrogen-fixing alga *Anabaena cylindrica* Lemm., observations on the growth of pure cultures have yielded information which appears to shed some light on the nature of the Myxophycean heterocyst. The more important results obtained are described and discussed in the following account.

## I. Methods

### (*a*) *Culture methods*

The isolation of *A. cylindrica* in pure culture and the culture methods used have been described in a previous paper (Fogg, 1942). As before, each stock culture was tested for contaminants before being used as a source of inoculum for experimental cultures.

In addition to the culture chamber of the type used by De (Fogg, 1942), subsequently referred to as culture chamber A, an apparatus similar to that described by Stoughton (1930) has been used and is designated as culture chamber B. The temperature of the former fluctuated between 21 and *c.* 27° C. with a mean temperature of 22·5° C. (this value being obtained by integration of thermograph records), whereas the latter was maintained at a temperature of 23·0 ± 0·3° C. Illumination in both chambers was for a period of 8 hr. daily. The light intensity in A, as determined by means of a Weston 'Lightometer' model E 703, varied from 120 to 160 foot-candles and that in B, from which daylight was excluded, was of the order of 800 foot-candles at a distance of 50 cm. from the lamp. To eliminate as far as possible the effects of any unequal illumination, the positions of the culture flasks within the chamber were changed at daily intervals.

### (*b*) *Estimation of growth rate*

The amounts involved being very small, the early period of growth of a culture could not conveniently be investigated by means of nitrogen or dry-weight determinations. The method of Bristol Roach (1926) has therefore been used. As adapted for use with *Anabaena*, it consisted in taking daily measurements of filament length per unit volume of medium. Since the diameter of the trichome was found to remain nearly constant in liquid culture, filament length has been taken as proportional to bulk of alga.

(1) *Culture vessels.* The form of culture vessel used was similar to that described by Bristol Roach except that 250 ml. Pyrex distilling flasks, fitted with a short length of pressure tubing and a screw clip instead of a stop-cock, were employed. Each flask, which contained 150 ml. of medium, was weighed before and after sterilization. Sterile distilled water was then added to make up for the loss which had occurred.

(2) *Inoculation.* A portion of material from a stock culture was shaken with sterile water for 15 min. A more uniform suspension was then obtained by filtration, this

process being carried out in a calcium chloride tube, the bulb of which contained a loose mass of glass wool. After the filament length per unit volume had been determined, sufficient of the filtered suspension was added to the experimental flasks to give the desired amount of inoculum. All the apparatus used in the preparation of the inoculum was sterilized in the autoclave prior to use.

(3) *Incubation.* The cultures were incubated in culture chamber B and were shaken thoroughly twice daily, when the lamps were switched on and off.

(4) *Sampling.* This was carried out in the manner described by Bristol Roach. Immediately after collection the samples were treated with a minute drop of saturated mercuric chloride solution to prevent further growth and were slightly acidified so that the precipitate which was present should be dissolved and not interfere with the process of measurement.

(5) *Estimation of mean filament length per unit volume of culture medium.* This was accomplished by means of a counting cell, $5 \times 2 \times 0{\cdot}05$ cm. in dimensions, of the type described by Chu (1942, p. 289), and a micrometer eyepeice. The cell was filled by means of a straight-sided pipette after thorough shaking of the filament suspension. Except in the very early stages of a culture, when it was not always possible, each estimate is based on measurements of a total of about one hundred filaments taken

Table 1. *Variation between replicate estimations of mean filament length per unit volume ($\mu$/mm.$^3$) of the same culture*

| | |
|---|---|
| 115<br>116 | Mean = 123·2 |
| 134·5<br>133 | $s$ = 12·4 |
| 106<br>134·5 | $s$ as percentage of the mean = 10·06 |

from at least two such samples. The main source of error, besides those invariably associated with the use of a counting cell, is due to the fact that the length of individual filaments varies greatly and that, occasionally, tangles of filaments are encountered the filament length of which it is impossible to determine with any degree of accuracy. Some idea of the error involved is given by results of replicate estimations on the same culture presented in Table 1. After about 8 days of growth, the adherence of the alga to the sides of the culture vessels and the formation of dense tangles of filaments rendered this method useless. A more or less uniform suspension of filaments could be obtained from older cultures by very vigorous shaking after the growth had been deteached from the sides of the flask by means of a rubber-tipped glass rod. This treatment, of course, involved destroying the culture. When it was necessary to estimate growth in older cultures by measurement of filament length, a large number of cultures was prepared and filament length was determined in sample cultures withdrawn at intervals.

### (*c*) *The determination of mean cell length and heterocyst frequency*

Mean cell length was determined by dividing the total length of several filaments by the number of cells of which they were composed. Heterocyst frequency is expressed as a percentage of the total number of cells. Counts and measurements were made on a sufficient number of filaments, selected at random, to give a total of about 400 cells.

That this gives sufficiently accurate results is shown in Table 2, which presents five estimates made on material from the same culture.

Table 2. *Variation in estimates of mean cell length* ($\mu$) *and heterocyst frequency* (%). *Replicate determinations on a single culture*

| No. of filaments | No. of cells | No. of heterocysts | Heterocyst frequency | Mean cell length |
|---|---|---|---|---|
| 14 | 389 | 19 | 4·88 | 3·38 |
| 15 | 472 | 23 | 4·87 | 3·20 |
| 12 | 404 | 20 | 4·95 | 3·39 |
| 9 | 393 | 20 | 5·09 | 3·35 |
| 10 | 367 | 18 | 4·90 | 3·32 |
| Mean | | | 4·94 | 3·33 |
| Standard deviation, *s* | | | 0·09 | 0·077 |
| *s* as percentage of mean | | | 1·8 | 2·3 |

### (*d*) *Chemical methods*

Total nitrogen was estimated, as in previous work (Fogg, 1942), by a micro-Kjeldahl method.

### (*e*) *Statistical methods*

The methods and symbols used are those given by Fisher (1938).

## II. Observations on the growth of *Anabaena cylindrica* in pure culture

### (*a*) *General observations*

When a limited amount of culture medium is inoculated with a suspension of *Anabaena* filaments (1–10$\mu$ per mm.$^3$ of medium) and exposed to suitable conditions of light and temperature, no growth is visible for a period of about 7 days. At the end of this time minute amounts of alga become apparent and subsequently spread rapidly. Growth is largely confined to that part of the surface of the flask covered by the medium, the filaments being arranged in a characteristic spiral fashion. The bulk of the medium usually remains relatively clear, although a certain amount of growth may eventually appear on its surface. After a time, the length of the period depending on the conditions of culture, the alga begins to turn yellow and finally dies and becomes colourless.

Some of the changes occurring in such cultures have been investigated, attention being directed chiefly to changes in morphology and growth rate. The results of a typical experiment, in which determinations of the nitrogen content of the alga in relation to filament length were also made, are given in Table 3. On account of the large amount of culture medium necessary in order that a sufficient quantity of alga might be available in the early stages of growth, replication was inconvenient and only one culture was examined at a time. The results obtained, however, are in agreement with those of more detailed but less comprehensive work and serve to illustrate the general trend of changes in *Anabaena* cultures.

Relative growth rate did not remain constant during the period of the experiment but fell as the cultures became older (see Fig. 1, in which the logarithm of filament length is plotted against time). The number of observations is not sufficient to indicate whether this fall was continuous or whether the relative growth rate remained constant

Table 3. *Changes in nitrogen/filament length (mg./μ × 10⁹),* mean cell length (μ) and heterocyst frequency (%), with time in cultures of* Anabaena cylindrica. *Filament lengths in μ/mm.³ of medium. Inoculum from 41-day-old stock culture.* 500 *ml. medium per flask. Incubation in culture chamber B*

| Age of culture in days | Filament length | N/filament length | Mean cell length | Heterocyst frequency |
|---|---|---|---|---|
| 0 | 28·4 | 0·400 | 3·49 | 2·8 |
| 5 | 93·3 | 0·489 | 3·10 | 0·0 |
| 8 | 339 | 0·295 | 2·92 | 1·9 |
| 11 | 544 | 0·276 | 3·32 | 4·9 |
| 16 | 1260 | 0·266 | 3·28 | 4·8 |
| 25 | 7640 | 0·299 | 3·51 | 4·0 |
| 35 | 14100 | 0·307 | 3·52 | 3·3 |

* The ratio nitrogen/filament length was determined by estimation of the total nitrogen present in the alga centrifuged down from a suspension containing a known length of filament. The accuracy of these estimations being largely dependent on the accuracy with which filament length was determined, sufficient filaments were measured to reduce the standard deviation to the order of 5%.

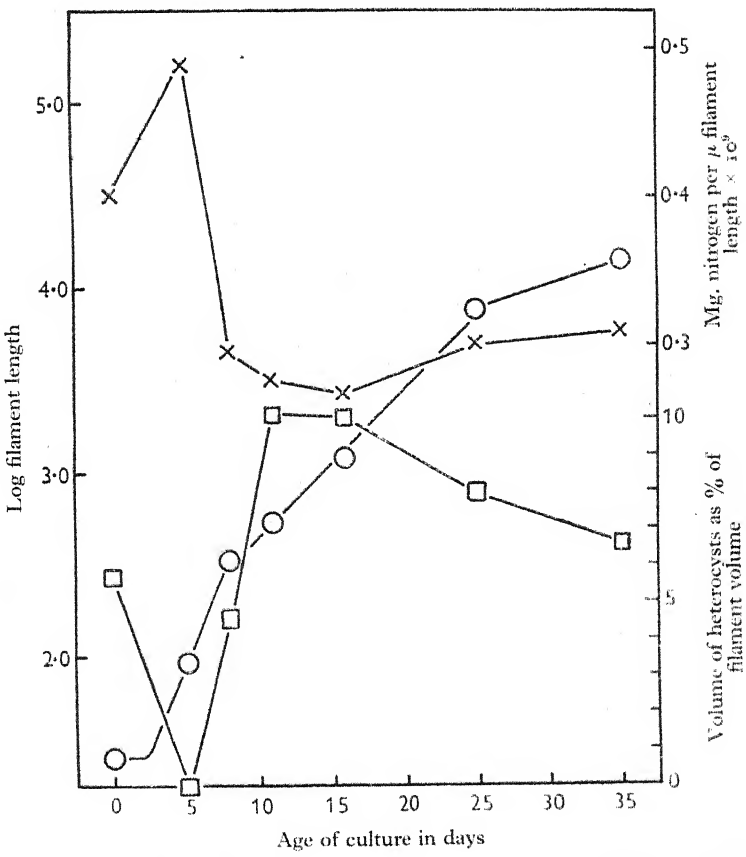


Fig. 1. Growth of *Anabaena cylindrica* Lemm. in culture. ——O——, logarithm of filament length in μ/mm.³ of medium; ——□——, volume of heterocysts as percentage of filament volume; —— × ——, mg. of nitrogen per μ filament length × 10⁹.

during certain phases of growth. Mean cell length decreased to a minimum about the eighth day and then rose again to a value of the order of that of the inoculum. Heterocyst frequency dropped abruptly within the first few days, subsequently rising to a high value during the period 11–16 days and then falling slightly.

The ratio nitrogen/filament length showed reciprocal changes to those of heterocyst frequency. Change in the value of this ratio might be due to variation in any of the following:

(1) mean diameter of the trichome;
(2) the amount of nitrogen-containing mucilage in the culture;
(3) the proportion of nitrogen per unit volume of cell material.

No fluctuations of mean trichome diameter were observed during this experiment. A difference of 0·8 $\mu$, which should easily have been detected, would be needed to account for the difference between the highest and lowest values of the ratio if it is assumed that the amount of nitrogen per unit volume of cell material remained constant. Secondly, it has never been possible to demonstrate the presence of mucilage in liquid cultures of *A. cylindrica* by means of stains such as methylene blue or ruthenium red or by means of diluted Indian ink. If, therefore, mucilage is present at all, it must be extremely diffuse and it is improbable that variation in its production could account for the observed fluctuations of the ratio. It thus appears that changes in the ratio nitrogen/filament length indicate corresponding changes in the amount of nitrogen per unit volume of cell material.

The correlation between heterocyst frequency and the nitrogen/filament length ratio is negative and statistically significant. In order to obtain a better measure of heterocyst formation the frequency has been converted to volume expressed as a percentage of total filament volume. To do this, mean heterocyst size, which remained roughly constant throughout the growth cycle of the alga, has been taken as 5·25 × 4·00 $\mu$, and the volume of normal cells has been calculated from the mean cell lengths as given in Table 3. It has been assumed that both normal cells and heterocysts are simple cylinders. The values so obtained for heterocyst frequency expressed as percentage by volume are given in Table 4.

Table 4. *Correlation between the nitrogen/filament length ratio and heterocyst frequency expressed as percentage by volume*

| N/filament length | % by volume of heterocysts |
|---|---|
| 0·400 | 5·65 |
| 0·489 | 0·00 |
| 0·295 | 4·58 |
| 0·276 | 10·10 |
| 0·266 | 10·00 |
| 0·299 | 7·93 |
| 0·307 | 6·60 |

$S(x^2)=0{\cdot}03984$, $S(y^2)=73{\cdot}8653$, $S(xy)=-1{\cdot}4888$,
$n=5$, $r=-0{\cdot}8676$, $P=$ between 0·02 and 0·01.

### (*b*) *The early period of growth*

The length of filament per unit volume of culture medium has also been used as a basis for estimating growth in the very early stages of a culture. Details of the method have been given on p. 164. The results of a typical experiment, together with parallel

observations on mean cell length and heterocyst frequency are given in Table 5 and Fig. 2. It will be seen that initially there was a lag period of about 1 day during which no increase

Table 5. *Growth of* Anabaena cylindrica *as determined by filament length measurements. Cultures inoculated from a 30-day-old liquid culture. Incubation in culture chamber B. Each result is the mean from three parallel cultures*

| Age of culture in days | Mean filament length ($\mu$/mm.$^3$) | Mean cell length ($\mu$) | Heterocyst frequency (%) |
|---|---|---|---|
| 0 | 1·96 | 4·40 | 3·9 |
| 1 | 1·71 | 3·50 | 3·7 |
| 2 | 3·44 | 3·05 | 1·4 |
| 3 | 7·12 | 3·13 | 1·0 |
| 4 | 16·8 | 3·08 | 0·3 |
| 5 | 32·7 | 3·03 | 4·75 |
| 6 | 59·2 | 3·08 | 5·9 |

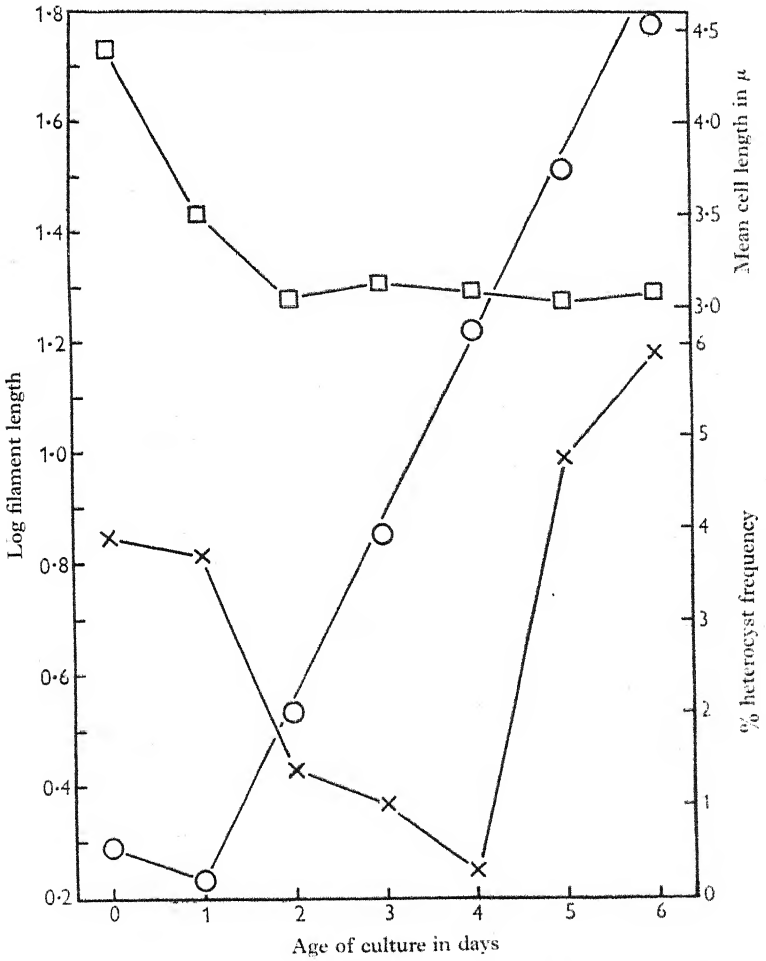


Fig. 2. Growth of *Anabaena cylindrica* Lemm. in the early stages of a culture. ——O——, logarithm of filament length in $\mu$/mm.$^3$ of medium; ——□——, mean cell length in $\mu$; —— × ——, percentage heterocyst frequency.

in filament length was evident. An apparent lag period might be caused by inoculation of an amount of alga smaller than that intended owing to errors in measurement of filament length and of volume of inoculum. The existence of a true lag period has, however, been checked in other cultures by direct counting. After the lag period followed a phase of rapid growth during which the relative growth rate remained constant. Relative growth rate may be derived from an expression of the form

$$R = \frac{\log_e B - \log_e A}{t},$$

where $A$ is the amount of alga originally present and $B$ the amount present after $t$ days. In this case $R$, calculated as the regression coefficient of the natural logarithm of mean filament length per unit volume on time in days, is $0{\cdot}749 \pm 0{\cdot}016$, that is, the bulk of the alga doubled in 22·2 hr. Mean cell length decreased rapidly during the first 2 days and thereafter remained constant at just over $3\mu$. The mean diameter of the trichome ($3{\cdot}4\mu$) did not alter appreciably. Heterocyst frequency decreased, reaching a minimum on the fourth day, when, however, numerous cells about to turn into heterocysts were observed. After this, heterocyst frequency increased rapidly to above its former value.

After the sixth day the filaments tended more and more to become aggregated in dense tangles and to adhere to the sides of the flask and the method became unreliable. It is consequently impossible to say whether the apparent falling off in relative growth rate observed, more particularly in other, similar, experiments, at the end of this period was real or whether it was due to increasing inaccuracy of the method.

### (*c*) *The lag period*

A certain amount of variation in the duration of the lag phase and in the relative growth rate in the exponential period following it has been observed. This variation appears to depend on the condition of the culture from which the inoculum was taken. A summary of data relating to control cultures in four different experiments is given in Table 6. Although these experiments were conducted at different times, the conditions

Table 6. *Variation in duration of lag period and in relative growth rate of cultures inoculated with* Anabaena cylindrica *from parent cultures of different ages. Incubation in chamber B*

| Nature of parent culture | Age of parent culture in days | Amount of inoculum $\mu$/mm.$^3$ | Duration of lag in days | $R$ during exponential phase | Standard deviation of $R$ |
|---|---|---|---|---|---|
| Liquid | 40 | 13·9 | 0 | 0·482 | ±0·020 |
| Agar | 18 | 2·0 | 0 | 0·468 | ±0·046 |
| Agar | 34 | 5·7 | 2 | 0·698 | ±0·061 |
| Agar | 55 | 9·2 | 2 | 0·716 | ±0·129 |

of culture were similar and the only variable (other than a small variation in the amount of inoculum) appears to have been the condition of the parent culture. While quantitative evidence regarding the growth rates of the parent cultures is lacking, this information suggests that material from an actively growing culture continues rapid growth without a preliminary lag period when inoculated into fresh medium. Under such circumstances, however, the relative growth rate is less than that in cultures inoculated from material in less active growth, in which there is a lag period.

An experiment has also been carried out in which the growth of inoculum derived at different times from the same culture has been determined (Table 7). These results show that the lag period lasted for about one day when the inoculum was derived from 20- to 40-day-old cultures. Inoculum from a 60-day-old, senescent, culture showed a lag period of nearly 2 days. The accuracy of the data available is not sufficient to show whether or not slow growth takes place during this lag phase. In each case $R$ remains constant for a period of 4–5 days after the commencement of rapid growth. There are no statistically significant differences in the values for $R$ for the different series, though the method was in general sufficiently sensitive to have revealed differences greater than 10%.

Table 7. *Growth, as determined by filament length, of inoculum derived at different times from a liquid culture of* Anabaena cylindrica *parallel to those of the experiment the results of which are given in Table* 8. *Each estimate is the mean of counts made on three cultures. Incubation in chamber B. Filament lengths in* $\mu$/*mm.*[2]

| Age of parent culture in days ... | 20 | 30 | 40 | 60 |
|---|---|---|---|---|
| $R$ for parent culture* ... ... | 0·308 | 0·119 | 0·046 | 0·034 |
| Age of culture in days | | | | |
| 0 | 1·96 | 1·96 | 1·96 | 1·96 |
| 1 | 2·23 | 1·71 | 2·18 | 2·10 |
| 2 | 4·98 | 3·44 | 6·05 | 2·20 |
| 3 | 12·0 | 7·12 | 6·00 | 4·50 |
| 4 | 22·8 | 16·8 | 18·25 | 8·1 |
| 5 | 45·3 | 32·7 | 30·6 | 18·5 |
| 6 | 77·0 | 59·2 | 70·0 | 42·5 |
| 7 | 104·4 | — | 99·4 | 76·2 |
| 8 | — | — | — | 111·1 |
| Duration of exponential growth (days) ... ... ... | 1–5 | 1–5 | 1–6 | 2–7 |
| $R$ ... ... ... ... | 0·754 | 0·749 | 0·666 | 0·723 |
| $s_R$ ... ... ... ... | ±0·027 | ±0·016 | ±0·060 | ±0·019 |

* During 10–20 days before subculturing.

### (*d*) *The later period of growth*

After about 14 days the amount of alga in a culture is sufficient to allow growth to be measured by dry weight or nitrogen determinations. Table 8 gives data from an experiment in which the former was used as an estimate of growth.* A gradual falling off in relative growth rate from an initial value of *c.* 0·119 occurred throughout the period of this experiment. Other experiments under similar conditions have shown, however, that $R$ usually remains constant from about the fourteenth day until the thirty-fifth day. At the end of the experiment the cultures were yellowish and senescent. Mean cell length fluctuated in no very regular manner but remained at a high value throughout the period being considered. Heterocyst frequency was at a maximum at 20 days, falling to a minimum at 40 days and subsequently rising again. Again heterocyst frequency and the nitrogen content of the alga tend to vary inversely but in this case the number of pairs of observations is insufficient to allow a statistically significant correlation to be

* Although the culture conditions, particularly light intensity, were somewhat different from those in the experiment described on p. 166 the results are essentially similar to those obtained in that experiment.

established. Spores have never been observed in liquid cultures to which no combined nitrogen has been added. In 60-day-old cultures a certain amount of cyanophycin became evident in the cells, otherwise the alga was devoid of conspicuous granular contents throughout the experimental period.

Table 8. *Growth of* Anabaena cylindrica *as estimated by dry-weight determinations. Inoculum from 63-day-old stock culture. Incubation in chamber A. Dry weights are corrected for the precipitate present in the medium and expressed in mg. per 50 ml. of culture medium*

| Age of culture in days | No. of cultures used per estimation | Dry weight | Mean cell length $\mu$ | Heterocyst frequency % | % nitrogen on dry-weight basis |
|---|---|---|---|---|---|
| 0 | — | — | 4·50 | 2·4 | — |
| 20 | 9 | 1·69 | 3·75 | 7·45 | 7·13 ± 0·50 |
| 30 | 6 | 5·53 | 4·40 | 3·9 | 7·64 ± 0·45 |
| 40 | 3 | 8·75 | 3·90 | 2·6 | 8·30 ± 0·67 |
| 60 | 4 | 17·35 | 4·40 | 3·75 | 7·27 ± 0·38 |

## III. Discussion

In summarizing these results, four main stages may be distinguished in the growth cycle of *A. cylindrica* under the conditions of culture in this investigation.

First, a period has been observed during which no marked increase in the bulk of the alga takes place. The duration of this lag depends on the condition of the parent culture, being approximately 2 days when the inoculum is derived from senescent material and less, or absent altogether, when it is taken from cultures in more active growth. This period is evidently occupied by a transition of the cells from a less active to a meristematic condition, since there is a decrease in mean cell length and, probably, an increase in the amount of nitrogen per unit volume of cell material. Heterocyst frequency remains unchanged during the lag phase.

Following this is a period of rapid, generally exponential, growth, during which a relative growth rate of the order of 0·45–0·70 is maintained for 5 days or more. The beginning of this phase is marked by a rapid decline in mean cell length, which then remains nearly constant at a relatively low value until it commences to rise again towards the end of the period. Heterocysts are almost absent just after the lag then their frequency rises abruptly to a high value. The cell nitrogen content drops, suddenly at first and then less rapidly, to a low value.

Later, when the relative growth rate has fallen to about 0·12, another period of exponential growth is found. Cell extension has occurred and mean cell length remains at a high value. Nitrogen content rises slowly, recovering from the depletion produced by the initial phase of rapid growth. Heterocyst frequency falls slowly. Although relative growth is small, actual growth is greatest during this period.

Finally comes a period of senescence marked by falling relative growth rate, rising heterocyst frequency, and eventually, yellowing and death.

Until more detailed information regarding the growth cycle of *Anabaena* is available, comparison with that of an alga such as *Chlorella* is perhaps unprofitable. The occurrence of a lag phase in *Anabaena* is, however, of interest since the majority of workers with

green algae (e.g. Pearsall & Loose (1937), with *Chlorella vulgaris*, and Bristol Roach (1926), with *Scenedesmus costulatus*) have found that such a phase does not normally occur in the forms studied by them. More recently, Pratt & Fong (1940) have observed a retardation of the early growth of *Chlorella vulgaris* when the inoculum is taken from older cultures. Such a lag seems to be essentially similar to that shown by *Anabaena* and by many bacteria such as *Bacterium coli* (see, for example, Penfold, 1914). The distinction in this respect between *Anabaena* and the Chlorophyceae does not appear to be fundamental.

Although there has been much speculation regarding the nature of heterocysts no satisfactory explanation of these structures has yet been advanced. The subject has lately been reviewed by Fritsch (1944), and it will suffice here to comment on two theories which have received support in recent years. It was once held that heterocysts are storage organs but this seems unlikely since their formation commences with the disappearance of reserve materials and no such substances are present in any quantity in the mature organ. Canabaeus (1929) has revived the theory in a somewhat different form by suggesting that heterocysts serve as receptacles for hypothetical enzymes responsible for a fermentation resulting in the production of gas-vacuoles. The presence of such enzymes in the heterocysts has, however, yet to be convincingly demonstrated, and this hypothesis gives no indication of the nature of the factors concerned in the formation of the structures in question. Since a certain relationship between heterocysts and spores can be observed and since the contents of the former occasionally germinate, it has been held that they represent archaic reproductive cells now largely functionless (Geitler, 1936). A spatial relationship to spores and a capacity for germination, however, are inadequate grounds on which to base a theory of the persistence of an aborted reproductive cell, a phenomenon for which there is no parallel in any other group of organisms.

Heterocysts are generally distributed more or less uniformly among the filaments of an alga and, although variation in salt concentration, for example, may affect their numbers (Canabaeus, 1929), their presence or absence does not appear to be determined directly by any particular environmental factor. Geitler (1936, p. 72) has drawn attention to the infrequency of the structures among actively dividing cells, remarking 'Es zeigt sich allgemein ein gewisser Antagonismus zwischen der Höhe der Teilungsfrequenz und Heterocystenbildung'. In the course of the present work a characteristic fluctuation in heterocyst numbers during the growth cycle of *A. cylindrica* has been observed. In this case also, it has been noticed that heterocyst frequency drops to a very low level during the initial period of rapid growth.

This relationship between heterocyst frequency and growth rate is not rigid. Thus, in the experiment described on p. 166, falling relative growth rate after the 11th day was accompanied by falling heterocyst numbers. An inverse correlation between heterocyst frequency and cell nitrogen content has, however, been found to hold. It would seem from this that heterocyst formation is most active when the alga is in a depleted condition. This agrees with results of microscopical investigations, which indicate that the structures in question have less dense contents than those of normal cells. Heterocysts present a rather empty appearance and, when mature, their protoplasm is devoid of chlorophyll and appears homogeneous. Storage products, notably cyanophycin, a nitrogenous reserve substance, are absent in any quantity, although the cellulose laid

down on the inner side of the cell wall has been regarded as a reserve substance by Geitler (1936). The cell wall is rigid and is generally separated from the cell contents by a more or less wide interval (Fritsch, 1904).

Comparison with an organism such as *Chlorella*, indicates that heterocyst formation may bear, in some respects, the same relation to the life cycle as does vacuolation in the green alga. At the end of a period of rapid growth the relative dry weight of the cells of *Chlorella* is at a minimum and it is at this stage that extensive vacuolation becomes evident (Pearsall & Loose, 1937). It is at the end of the initial period of rapid growth, when the nitrogen content of the alga is at a minimum, that heterocysts are produced most abundantly in *Anabaena*. One of the chief characteristics of vacuoles is that they are regions of salt accumulation. The little evidence available regarding salt accumulation in heterocysts indicates that this also occurs in these organs. Lloyd (1925), using the cobaltinitrite method of Macallum (1905) to determine the localization of potassium in blue-green algae, observed that the potassium precipitate was nearly always present in the pores of the heterocysts of *Cylindrospermum*, whereas its occurrence in normal cells was sporadic. This has also been found to be the case in *Anabaena cylindrica*, in which the potassium precipitate constantly appeared in a region of the heterocyst adjacent to the pore and only sparsely and in an irregular manner elsewhere. Bearing in mind Lloyd's criticism of the cobaltinitrite reagent as a means of determining exactly the localization of potassium in the living cell, it appears that potassium, at least, is accumulated mainly in the heterocysts of these forms and support is given to the thesis that these organs are in some respects analogous to vacuoles.

In view of these considerations it is tentatively suggested that the formation of heterocysts may be the result of processes similar to those leading to vacuole formation in plants other than blue-green algae and that heterocysts and vacuoles may have certain characteristics, such as that of salt accumulation, in common. Cell extension, involving as it does an increase in cell volume without a parallel increase in non-aqueous constituents of the protoplasm, must result either in an increase in the vacuome in relation to the cytoplasm or in a dilution of the cytoplasm. Minute vacuoles in Myxophycean cells have been reported by Guilliermond (1926) but an extensive vacuome of the type met with in other plants is absent except in moribund material (Geitler, 1936, p. 12). Cell extension in the Myxophyceae would appear to involve an increase in the aqueous phase of the protoplasmic colloids rather than an increase in the size of the vacuoles. It is suggested that a cell begins to change into a heterocyst when this dilution of its cytoplasm has reached a limiting stage. In a form such as *Anabaena*, in which growth is diffuse but in which the cells of a filament are never all in the same stage of division, it is easy to see how certain cells may become depleted more rapidly than others, resulting in a scattered distribution of heterocysts. It is perhaps significant that in the Oscillatoriaceae, in which cell division proceeds simultaneously in all the cells of a filament and in which, therefore, no one cell is likely to become more depleted than another, heterocysts are absent.

## IV. SUMMARY

1. A study has been made of the growth in pure culture of *Anabaena cylindrica* Lemm., attention being chiefly directed to growth rate and to changes in certain morphological characteristics.

2. In cultures of the type used, four main phases of growth have been distinguished:

(*a*) a lag period, the duration of which depends on the condition of the parent culture; (*b*) a period of rapid exponential growth; (*c*) a period after cell extension has occurred of slower relative growth which may also be exponential; and (*d*) senescence.

3. Heterocyst numbers show characteristic fluctuations during the growth cycle, the frequency of these structures being least at the beginning of period (*b*) and highest towards its end. Heterocyst frequency shows a negative correlation with cell nitrogen content.

4. The nature of heterocysts is discussed in the light of these results.

In conclusion I wish to express my thanks to Prof. F. E. Fritsch, F.R.S., for advice and criticism concerning this work.

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# REVIEWS

*An Introduction to Pollen Analysis.* By G. ERDTMAN. $10\frac{1}{2} \times 7$ in. Pp. 239 with 46 plates and illustrations. New Series of Plant Science Books, vol. XII. Chronica Botanica, Waltham, Mass., U.S.A., Messrs Wm Dawson and Sons, London. Price $5.00.

As the imagination of Lagerheim and the scientific insight and tenacity of von Post forged the weapon of pollen analysis, so the eagerness and facility in expression of G. Erdtman led to his publicizing in English the methods of his Scandinavian colleagues. He thereby has done great service, and now our debt to him is deepened by the publication of the first text-book of pollen analysis, which is written in English and well produced by Chronica Botanica.

The most substantial part of the book is the description of pollen- and spore-morphology for a wide range of species, mostly north-west European, but including a scatter of forms from the New World and the Antipodes. These are well illustrated, and the author has interjected comments on the most significant results so far met with in the elucidation of the history of the various types. Thus, for instance, in dealing with the pollen of the Ericaceae, he mentions the contribution of pollen analysis to solution of the problems of the status of the north-west German heaths, and, in dealing with Gramineae, indicates how recognition of the cereal pollen type throws light on the prehistoric cultivation of grain. Twenty-eight full-page plates illustrate pollen and spore morphology, and they will prove invaluable.

The author's attempts to improve our capacity for species recognition are very welcome, but he goes further in his assertions of what is possible in this direction than seems warranted by present experience. Thus in asserting that *Myrica gale* pollen is distinguishable from that of *Corylus avellana* by its having no 'arci' (curved bands of thickening between the germ-pores), he ignores the practical difficulty of seeing arci in *Corylus* itself, difficulties so great that the arci are in fact only now reported. It is hard also to see what is gained by saying that these arci are merely 'ornament'.

Dr Erdtman has set out to give the widest possible interpretation to the term 'pollen analysis', and to exclude none of its possible developments. It will therefore be found that he alludes to a fascinating number of topics, from the well-known uses of the method in relation to bog stratigraphy and archaeology, to pollen analysis in honey and glacier ice, to collection of pollen from the air by sticky slides or by vacuum cleaner from the air over the Atlantic, to applications in the study of hay fever, to spore counts in coal seams, to comparison of surface catches of pollen in different plant communities, to identification of Tertiary pollen, and so forth. Dr Erdtman's brother contributes a chapter on the chemistry of peat which is very satisfactory as a basis for consideration of the problems of preservation of pollen-grain membranes and their extraction from a peaty matrix.

An immense and most useful list of literature is given in relation to all these topics, and this widespread acquaintance with the world's literature enhances the book's value, although at the same time perhaps leading to the dangerous conclusion that the author's summation of that literature may be regarded as final and adequate. It is important that this should not be thought, for the attitude displayed to many problems is insufficiently critical, and one misses an objective summing up of evidence in debatable issues, whilst the tone tends to be authoritative.

Dr Erdtman's own technique of chlorination and acid hydrolysis (surely not correctly called 'acetolysis') for the removal of lignin and cellulose, so as to concentrate the pollen grains for counting, has proved of very great value, but one cannot help wondering why his peat samples must all be dehydrated and powdered before this treatment. Mechanical dispersion of the wet peat or disintegration in hot dilute alkali saves much time and allows straining off of fruits, seeds, and similar large material for identification of the peat type. Dehydration *may* be better, but there is no evidence of this.

It may well be felt in the Scandinavian countries where pollen analysis technique has been so whole-heartedly and successfully devoted to the solution of the problems of quaternary geology, that this, the outstanding achievement of the method, gets less than due attention in the book.

Despite the limitations mentioned, this is a very timely book, certain to engage the attention of a very wide scientific public, attracted by the remarkable potentialities of the new field of investigation. They will find a good style of English, useful and copious illustration, extensive bibliographies and valuable working instructions.

H. GODWIN

*The Cytoplasm of the Plant Cell.* By ALEXANDRE GUILLIERMOND. Trans. by L. R. Atkinson. 10½ in. by 7 in. Pp. 247, with 156 figures in the text. Chronica Botanica, Massachusetts, 1941, $4·75; and Wm. Dawson and Sons, London. 27*s*.

This book is a translation of an unpublished French manuscript. It contains a useful general description of the cytoplasm of plant cells, but the bulk of it is concerned with two controversies in which the author has played a distinguished part. These concern the relationship of plastids to mitochondria,[1] and the 'vacuome' theory of Dangeard.

In 1910–11 three independent investigators, Pensa, Lewitsky and Guilliermond, showed that the plastids of phanerogams develop from meristematic elements having the appearance and properties of mitochondria. As Guilliermond insists, this is no mere chance resemblance. Not only in form, but also in physical properties and chemical composition, there is a close affinity between mitochondria on the one hand and the structures that will later on become plastids on the other. In most cases, however, the embryonic plastids are distinguished by being larger; and this fact, taken in conjunction with the obvious separateness of mitochondria from chloroplasts in Algae, has caused Guilliermond to write of 'the duality of the chondriome'. He thinks that although embryonic plastids have a genuine resemblance to mitochondria, so that both may be grouped together as 'chondriome', yet mitochondria only give rise to mitochondria and plastids to plastids. Now in certain cases the two are indistinguishable, as Guilliermond unequivocally allows on p. 92. It is therefore surprising that he should conclude so very definitely on pp. 113–14 that there is no genetic connexion between them. Who can be certain, for instance, that in the chondriome of the foliar primordia of *Elodea canadensis* certain elements are already predetermined to remain mitochondria, while others, in no way distinguishable, must inevitably develop into chloroplasts? Guilliermond has himself shown how similar the two categories of objects are. Perhaps he does not push his conclusions quite far enough. It may be that plastids in some cases develop from mitochondria.

Guilliermond has brought strong arguments against the vacuome theory of Dangeard. He has shown that, although the vacuolar system of meristematic cells often resembles a group of mitochondria, yet the resemblance is illusory and not indicative of chemical affinity or genetic continuity. He has brought forward evidence that vacuoles are not necessarily self-perpetuating cell elements. Further, he denies that there is any one particular substance, corresponding to the 'metachromatin' of Dangeard, universally present in plant vacuoles. The contents of vacuoles, he insists, are extremely diverse.

In considering the question whether the Golgi element of animal cells corresponds to the vacuoles of plants, the author boldly and justifiably denies that the Golgi element is sufficiently well characterized by morphological or chemical criteria to be regarded as necessarily a single entity. Very different objects may reduce osmium tetroxide or silver nitrate in the standard Golgi techniques, without affording proof that those objects are homologous. This is a refreshing outlook, of which animal cytologists would do well to take note.

Dealing as it does with some of the most disputed questions of cytology, the book is a model of how scientific controversies should be conducted. It is also a model of clear exposition. One may disagree, but one cannot fail to understand. The absence of a proper subject index is regrettable, and there are a few points that one may query, especially some unimportant ones in connexion with the Golgi element and a dogmatic statement that the function of the pulsating vacuoles of flagellate algae is 'unknown'. Some readers, too, will wonder how Guilliermond can be so sure that certain objects in cells are 'living' and others not, and perhaps too much attention is paid to 'coacervates'. These, however, are not matters that seriously affect the chief problems discussed in the book. Prof. Guilliermond has made a valuable contribution to our knowledge of the cytoplasm, which will be appreciated by students of both plant and animal cells.

JOHN R. BAKER

[1] In this review the word *mitochondria* is used for what Guilliermond calls *chondriosomes*.

*Wild Flowers in Britain*. By GEOFFREY GRIGSON. (Britain in Pictures Series.) 48 pp., with 12 plates in colour and 22 illustrations in black and white. London: William Collins. 1944. Price 4*s*. 6*d*.

This addition to the Britain in Pictures series is as usual copiously illustrated with reproductions, some in colour and some in black and white. The coloured plates are, with a few pleasing exceptions (such as the Yellow Flag from Redouté's *Les Liliacées* and the Greater Celandine from Baxter's *British Flowering Plants*), rather disappointing. Slight inaccuracies in register have given a blurred outline and the colours are often muddy. By contrast the black and white reproductions, especially those from Curtis's *Flora Londinensis*, are excellent. In particular, the Fritillary (p. 14), the Coltsfoot (p. 15) and the Lords and Ladies (p. 31), have lost little of their outstanding beauty.

Every professional botanist should read what Mr Grigson has to say, for Mr Grigson holds that it is for his own delight and that of his fellow-amateurs that wild flowers grow in Britain, not for the small hand of 'learned botanists'. And though many of that band may dislike the out-moded teleology of the claim, they can hardly deny the underlying truth, nor fail to be made humble by it.

Mr Grigson's love for our wild flowers is sensitive and complex, and he writes with a captivating sincerity. Early in the book there is a quotation from Samuel Palmer, a passage of arresting appropriateness: '. . . all the very finest original pictures, and the topping things in nature, have a certain quaintness by which they partly affect us; not the quaintness of bungling—the queer doings of a common thought; but a curiousness in their beauty, a salt on their tails, by which the imagination catches hold on them.' That is the keynote of the whole book, Mr Grigson likes fumitory because it is a lovely plant, because it is so aptly called 'earth-smoke', and because Prof. Salisbury thinks it may have been introduced by the Megalith builders. He likes *Silene quinquevulnera* for its beauty and for the name Linnaeus gave it. He devotes a whole chapter to fritillary, deadly nightshade and henbane, and finds in Herb Paris much of what he looks for in a wild flower. He thinks flowers should be picked—in moderation—and that some flowers, such as fritillaries, must be picked before they yield up their full beauty. 'The right of children—and your right and mine—to pick flowers is worth more to humanity than the preservation of a rare plant', and 'if excellent plants threaten to become rare, they should, where it is possible, be reseeded and kept going'. Here Mr Grigson reveals his most furious disagreement with botanists. Nature should not be left alone just to provide them with a private dominion. May be, but Mr Grigson would find it hard to admire fritillaries with the sun shining through them if he had to rely on those meadows near Oxford where they were once so abundant and where now hardly a flower is allowed to open. He might then be glad that an Oxford College restricts the rights of children and preserves part at least of one fritillary meadow for all to enjoy. And may the botanist not claim some special rights? May he not claim that all lose something of value when his search for the facts of the coming and going of plants is hindered? There are surely ways in which these claims can be reconciled. Judicious restriction in certain reserved areas will maintain reasonable rights to the enjoyment of our rarer wild flowers; and children might broadcast seeds of exotic garden plants but not of native British plants. If rare native plants threatened with extinction are to be aided by reseeding, let botanists be consulted first, and, most important, let botanists know *exactly* what has been done—the source of the seed and exact date and place of sowing.

Mr Grigson has evidently read widely in botanical literature. He knows much about geographical distribution and plant dispersal, and something of ecology. But there are fields of scientific botany in which he appears never yet to have wandered. He would, for instance, find much to interest him, much that has the requisite saltiness, in the recent findings of the peat analysts and cyto-geneticists.

Mr Grigson ends with a word of pleasure at the initiation of the Biological Flora and an appeal for a 'popular guide planned in the same way . . . a book written by an expert who is not emotionally dead to all excellence in plants, and not above realizing that to popularize is one of the points of having scientific method and research'. There is a serious challenge. Anyone who feels moved to accept it should not fail first to read this book with care, and should aim at writing in a manner equally lively, thoughtful, and gracious.

A. R. CLAPHAM

*Problems in Tree Nutrition.* By M. C. RAYNER and W. NEILSON-JONES. $5\frac{1}{2} \times 8\frac{1}{2}$ in. Pp. 184, 27 plates, 11 text-figures. London: Faber and Faber. 1944. 12*s.* 6*d.* net.

The opportunity of publishing a collected series of papers usually comes only to the very distinguished at the end of a life's work, and it is an honour which any author would appreciate. The papers here collected are:

M. C. Rayner, *Forestry*, **8**, 1934, pp. 96–125: 'Mycorrhiza in relation to forestry. I. Researches on the genus *Pinus* with an account of experimental work on a selected area.'

M. C. Rayner, *Forestry*, **9**, 1935, pp. 154–5: 'Mycorrhizal associations in Scots pine.'

The above two papers form Chapter II of the book.

M. C. Rayner, *Forestry*, **10**, 1936, pp. 1–22: 'The mycorrhizal habit in relation to forestry. II. Organic composts and the growth of young trees.' Forms Chapter III.

M. C. Rayner, *Forestry*, **13**, 1939, pp. 19–35: 'The mycorrhizal habit in relation to forestry. III. Organic composts and the growth of young trees.' Forms Chapter IV.

M. C. Rayner and I. Levisohn. *Forestry*, **15**, 1941, pp. 1–36: 'The mycorrhizal habit in relation to forestry. IV. Studies in mycorrhizal response in *Pinus* and other conifers.' Forms Chapter V.

W. Neilson-Jones, *J. Agric. Sci.* **31**, 1941, pp. 379–411: 'Biological aspects of soil fertility.' Forms Chapter VI.

No important changes have been made in the text except for the amalgamation of the first two papers. An introductory first chapter has been added.

The work began on the heaths on Bagshot Beds near Wareham, Dorset, where sowings of *Pinus* spp. had given poor results. As has so often been found, vigorous healthy growth seemed to be correlated with profuse formation of mycorrhiza. Improvement of growth followed inoculation of seed beds with either pure cultures of mycorrhizal fungi or with humus from thriving pine stands. Addition of various composts stimulated growth greatly, but to an extent which varied surprisingly with the origin of the compost. Addition of compost and mycorrhizal inoculation together sometimes produced better growth than compost alone. Partially sterilized soil from a thriving pine stand grew poor seedlings, but inoculation with mycorrhizal fungus restored its fertility. Finally, Neilson-Jones demonstrated that a volatile toxin was formed by biological activity in the heath soil, especially under anaerobic conditions. This toxin prevented fungal growth and was very injurious to phanerogams.

Thus the importance of the work far exceeds that of its practical application to the cultivation of conifers. The problems studied have bearings on the wider aspects of plant nutrition, the use of manures in agricultural practice; the ecology of heath vegetation and of the higher fungi. The proof of the existence of toxins is of special importance since their existence has so often been suspected. The facts relating to their origin, destruction and effect on micro-organisms illuminate many problems of soil fertility. Accordingly, many botanists should be glad of the opportunity of obtaining these papers in such a convenient and well-reproduced form, especially since they were mostly published in a Journal with relatively small circulation.

It is a pity that the majority of the papers are—at least in the reviewer's experience—exceptionally difficult to follow. The major facts are fairly clear, but there are too many half-hidden facts and veiled statements. Perhaps Dr Rayner in writing for foresters had underestimated their capacity to assimilate details and has not realized their love of figures and statistics. At any rate, results are too often given in the form of photographs of selected pots of seedlings, or even of single seedlings and, when mean sizes are given, there is usually no indication of the number of plants which they represent nor of their variability. Reading between the lines it is obvious that Dr Rayner and her helpers have accumulated a wealth of detailed and laborious observations of which no account has yet been published. There are many claims made in her papers for which adequate evidence was not offered at the time they were written. Looking back, now that the series of papers is complete, one can see that many of the claims were justified, but the practice of making such unsupported statements does not win the confidence of the critical reader.

There is much repetition of statements of this kind. Thus on p. 52, Chapter II, we are told that 'Attention has been already directed to a phenomenon observed in all successful inoculation experiments . . . namely the fact that the subsequent stimulus to growth *precedes* mycorrhiza formation, is, indeed, directly responsible for it, because it promotes . . . the production of rootlets destined to become mycorrhiza.' In fact, the only previous information on this very important detail appears to be on p. 50 where we read that 'There is evidence that improved growth, in particular a great impetus to root development . . . precedes any possible effect due to mycorrhizal activity. . . .

*If* (italics mine) this stimulation of growth can be correlated directly with the activities of specific mycorrhiza-formers in the substrate previous to their symbiotic association with the roots....' We are not told what the evidence is and Dr Rayner *appears* to suggest some doubt as to the fact, although it is subsequently repeated in the summary to the paper. Many similar unsupported statements relating to soil toxins were not vindicated until the publication of Neilson-Jones's paper. Many physiologists will feel that the contention that the action of composts is not 'mainly due to an increased supply of directly available nutrients', and 'differs essentially from that of ordinary manures', is not adequately supported, especially as they will note that as far as can be ascertained from the analyses given, their effect varies with their phosphate content, and the most effective compost has the highest content of both phosphate and nitrogen. Moreover, the dressings used were not light, and the experiments designed to meet these criticisms, described in Chapter IV, though very interesting, are scarcely conclusive, since a decrease in root-shoot ratio would result from an improved supply of soil nutrients. Probably a marshalling of all the available evidence, together with a clearer definition of 'ordinary' manurial action, would fully substantiate Dr Rayner's claim.

The difficulty of following the papers is increased by failure to separate clearly the observed facts from hypotheses and theories, and by an involved style which often leaves the precise meaning of a sentence uncertain. Readers will wonder whether an 'effect' can be 'beneficient' whether a 'conclusion' can be 'deduced' and what is meant by 'the slowing down of a growth reaction'.

Should the reviewer seem unduly harsh in his criticisms, it must be remembered that he is merely stating his personal experience with the papers. Moreover, the scientist is essentially sceptical and incredulous, demanding proof for every claim, so that he may build on a firm foundation. The work described in this series of papers is undoubtedly important, and that is all the more reason for demanding that the foundation be firm.

E. W. JONES

# ERRATUM

Vol. 43, No. 1, 1944, p. 77

9th line of the Review *Die Ostgrenze Fennoskandiens*:

*for* east *read* west

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